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APPLICATION NUMBER: *09/255,794*
FILING DATE: *February 23, 1999*
PCT APPLICATION NUMBER: *PCT/US00/04336*

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UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 CFR 1.53(b))	Attny Docket No. PF343P2	Total Pages: 189
	First Named Inventor or Application Identifier: YU et al.	
	Date: February 23, 1999	

APPLICATION ELEMENTS (See MPEP chapter 600 concerning utility patent application contents.)	Assistant Commissioner for Patents *Address to:* Box Patent Application Washington, D.C. 20231
1. ☒ Fee Transmittal Form	6. ☐ Microfiche Computer Program (appendix)
2. ☒ Specification [total pgs.: 166]	7. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
(preferred arrangement set forth below)	a. ☐ Computer Readable Copy
- Descriptive title of the Invention	
- Cross References to Related Applications	
- Statement Regarding Fed Sponsored R&D	b. ☐ Paper Copy (identical to computer copy)
- Reference to Microfiche Appendix	
- Background of the Invention	c. ☐ Statement verifying identity of above copies
- Brief Summary of the Invention	
- Brief Description of the Drawings (if filed)	Accompanying Application Parts
- Detailed Description	8. ☐ Assignment Papers (cover and document(s))
- Claim(s)	9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attny
- Abstract of the Disclosure	10. ☐ English Translation Document
3. ☒ Drawing(s): 1A-12B Total Sheets 19	11. ☐ Information Disclosure Statement/PTO-1449
4. ☒ Oath or Declaration (unexec) Total pages 2	☐ Copies of IDS Citations
a. ☐ Newly executed (original or copy)	12. ☐ Preliminary Amendment
b. ☐ Copy from a prior application (1.63(d)) (for con/div with Box 17 completed)	13. ☒ Return Receipt Postcard (MPEP 503) (should be specifically itemized)
i. ☐ Deletion of Inventors (signed statement attached deleting inventor(s) named in prior app.)	14. ☐ Small Entity Statement(s) ☐ Statement filed in prior application, status still proper and desired
5. ☒ Incorporation by Reference (with Box 4) The entire disclosure of the prior application(s) recited in Box 17, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.	15. ☐ Certified copy of priority document(s)
	16. ☐ Other:

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:

☐ Continuation ☐ Divisional ☒ Continuation-in-Part (CIP) of Serial No. 09/005,874, filed January 12, 1998, which is a continuation-in-part of copending applications Serial No. US 60/036,100 filed January 14, 1997, and PCT/US96/17957 filed October 25, 1996.

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		Date

Neutrokine-α and Neutrokine-α Splice Variant

This application is a continuation-in-part of copending application Serial No. 09/005,874, filed January 12, 1998, which is a continuation-in-part of copending applications Serial No. US60/036,100 filed January 14, 1997 and PCT/US96/17957 filed October 25, 1996, each of which is herein incorporated by reference in its entirety.

The present invention relates to a novel cytokine which has been designated Neutrokine-α ("Neutrokine-α"). In addition, an apparant splicing variant of Neutrokine-α has been identified and designated Neutrokine-αSV. In specific embodiments, the present invention provides nucleic acid molecules encoding Neutrokine-α and Neutrokine-αSV polypeptides. In additional embodiments, Neutrokine-α and Neutrokine-αSV polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same.

Related Art

Human tumor necrosis factors (TNF-α) and (TNF-β, or lymphotoxin) are related members of a broad class of polypeptide mediators, which includes the interferons, interleukins and growth factors, collectively called cytokines (Beutler, B. and Cerami, A., *Annu. Ret. Immunol.*, 7:625-655 (1989)). Sequence analysis of cytokine receptors has defined several subfamilies of membrane proteins (1) the Ig superfamily, (2) the hematopoietin (cytokine receptor superfamily and (3) the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily (for review of TNF superfamily see, Gruss and Dower, *Blood 85(12)*:3378-3404 (1995) and Aggarwal and Natarajan, *Eur. Cytokine Netw.*, *7(2)*:93-124 (1996)). The TNF/NGF receptor superfamily contains at least 10 difference proteins. Gruss and Dower, *supra*. Ligands for these receptors have been identified and belong to at least two cytokine superfamilies. Gruss and Dower, *supra*.

Tumor necrosis factor (a mixture of TNF-α and TNF-β) was originally discovered as a result of its anti-tumor activity, however, now it is recognized as a pleiotropic cytokine capable of numerous biological activities including apoptosis of some transformed cell lines, mediation of cell activation and proliferation and also as playing important roles in immune regulation and inflammation.

To date, known members of the TNF-ligand superfamily include TNF-α, TNF-β (lymphotoxin-α), LT-β, OX40L, Fas ligand, CD30L, CD27L,

CD40L and 4-IBBL. The ligands of the TNF ligand superfamily are acidic, TNF-like molecules with approximately 20% sequence homology in the extracellular domains (range, 12%-36%) and exist mainly as membrane-bound forms with the biologically active form being a trimeric/multimeric complex. Soluble forms of the TNF ligand superfamily have only been identified so far for TNF, LT-β, and Fas ligand (for a general review, see Gruss, H. and Dower, S.K., *Blood, 85(12)*:3378-3404 (1995)), which is hereby incorporated by reference in its entirety. These proteins are involved in regulation of cell proliferation, activation, and differentiation, including control of cell survival or death by apoptosis or cytotoxicity (Armitage, R.J., *Curr. Opin. Immunol. 6*:407 (1994) and Smith, C.A., *Cell 75*:959 (1994)).

Tumor necrosis factor-alpha (TNF-a; also termed cachectin; hereinafter "TNF") is secreted primarily by monocytes and macrophages in response to endotoxin or other stimuli as a soluble homotrimer of 17 kD protein subunits (Smith, R.A. *et al., J. Biol. Chem. 262*:6951-6954 (1987)). A membrane-bound 26 kD precursor form of TNF has also been described (Kriegler, M. *et al., Cell 53*:45-53 (1988)).

Accumulating evidence indicates that TNF is a regulatory cytokine with pleiotropic biological activities. These activities include: inhibition of lipoprotein lipase synthesis ("cachectin" activity) (Beutler, B. *et al., Nature 316*:552 (1985)), activation of polymorphonuclear leukocytes (Klebanoff, S.J. *et al., J. Immunol. 136*:4220 (1986); Perussia, B., *et al., J. Immunol. 138*:765 (1987)), inhibition of cell growth or stimulation of cell growth (Vilcek, J. *et al., J. Exp. Med. 163*:632 (1986); Sugarman, B.J. *et al., Science 230*:943 (1985); Lachman, L.B. *et al., J. Immunol. 138*:2913 (1987)), cytotoxic action on certain transformed cell types (Lachman, L.B. *et al.*, supra; Darzynkiewicz, Z. *et al., Canc. Res. 44*:83 (1984)), antiviral activity (Kohase, M. *et al., Cell 45*:659 (1986); Wong, G.H.W. *et al., Nature 323*:819 (1986)), stimulation of bone resorption (Bertolini, D.R. *et al., Nature 319*:516 (1986); Saklatvala, J., *Nature 322*:547 (1986)), stimulation of collagenase and prostaglandin E2 production (Dayer, J.-M. *et al., J. Exp. Med. 162*:2163 (1985)); and immunoregulatory actions, including activation of T cells (Yokota, S. *et al., J. Immunol. 140*:531 (1988)), B cells (Kehrl, J.H. *et al., J. Exp. Med. 166*:786 (1987)), monocytes (Philip, R. *et al., Nature 323*:86 (1986)), thymocytes (Ranges, G.E. *et al., J. Exp. Med. 167*:1472 (1988)), and stimulation of the cell-surface expression of major histocompatibility complex (MHC) class I and class II molecules (Collins, T. *et al., Proc. Natl. Acad. Sci. USA 83*:446 (1986); Pujol-Borrel, R. *et al., Nature 326*:304 (1987)).

TNF is noted for its pro-inflammatory actions which result in tissue injury, such as induction of procoagulant activity on vascular endothelial cells (Pober, J.S. *et al., J. Immunol. 136*:1680 (1986)), increased adherence of neutrophils and lymphocytes (Pober, J.S. *et al., J. Immunol. 138*:3319 (1987)), and stimulation of the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells (Camussi, G. *et al., J. Exp. Med. 166*:1390 (1987)).

Recent evidence implicates TNF in the pathogenesis of many infections (Cerami, A. *et al., Immunol. Today 9*:28 (1988)), immune disorders, neoplastic pathology, e.g., in cachexia accompanying some malignancies (Oliff, A. *et al., Cell 50*:555 (1987)), and in autoimmune pathologies and graft-versus host pathology (Piguet, P.-F. *et al., J. Exp. Med. 166*:1280 (1987)). The association of TNF with cancer and infectious pathologies is often related to the host's catabolic state. A major problem in cancer patients is weight loss, usually associated with anorexia. The extensive wasting which results is known as "cachexia" (Kern, K. A. *et al. J. Parent. Enter. Nutr. 12*:286-298 (1988)). Cachexia includes progressive weight loss, anorexia, and persistent erosion of body mass in response to a malignant growth. The cachectic state is thus associated with significant morbidity and is responsible for the majority of cancer mortality. A number of studies have suggested that TNF is an important mediator of the cachexia in cancer, infectious pathology, and in other catabolic states.

TNF is thought to play a central role in the pathophysiological consequences of Gram-negative sepsis and endotoxic shock (Michie, H.R. *et al., Br. J. Surg. 76*:670-671 (1989); Debets, J. M. H. *et al., Second Vienna Shock Forum*, p.463-466 (1989); Simpson, S. Q. *et al., Crit. Care Clin. 5*:27-47 (1989)), including fever, malaise, anorexia, and cachexia. Endotoxin is a potent monocyte/macrophage activator which stimulates production and secretion of TNF (Kornbluth, S.K. *et al., J. Immunol. 137*:2585-2591 (1986)) and other cytokines. Because TNF could mimic many biological effects of endotoxin, it was concluded to be a central mediator responsible for the clinical manifestations of endotoxin-related illness. TNF and other monocyte-derived cytokines mediate the metabolic and neurohormonal responses to endotoxin (Michie, H.R. *et al., N. Eng. J. Med. 318*:1481-1486 (1988)). Endotoxin administration to human volunteers produces acute illness with flu-like symptoms including fever, tachycardia, increased metabolic rate and stress hormone release (Revhaug, A. *et al., Arch. Surg. 123*:162-170 (1988)). Elevated levels of circulating TNF have also been found in patients suffering from Gram-negative sepsis (Waage, A. *et al., Lancet 1*:355-357 (1987):

Hammerle, A.F. *et al., Second Vienna Shock Forum* p. 715-718 (1989); Debets, J. M. H. *et al., Crit. Care Med. 17*:489-497 (1989); Calandra, T. *et al., J. Infec. Dis. 161*:982-987 (1990)).

Passive immunotherapy directed at neutralizing TNF may have a beneficial effect in Gram-negative sepsis and endotoxemia, based on the increased TNF production and elevated TNF levels in these pathology states, as discussed above. Antibodies to a "modulator" material which was characterized as cachectin (later found to be identical to TNF) were disclosed by Cerami *et al.* (EPO Patent Publication 0,212,489, March 4, 1987). Such antibodies were said to be useful in diagnostic immunoassays and in therapy of shock in bacterial infections. Rubin *et al.* (EPO Patent Publication 0,218,868, April 22, 1987) disclosed monoclonal antibodies to human TNF, the hybridomas secreting such antibodies, methods of producing such antibodies, and the use of such antibodies in immunoassay of TNF. Yone *et al.* (EPO Patent Publication 0,288,088, October 26, 1988) disclosed anti-TNF antibodies, including mAbs, and their utility in immunoassay diagnosis of pathologies, in particular Kawasaki's pathology and bacterial infection. The body fluids of patients with Kawasaki's pathology (infantile acute febrile mucocutaneous lymph node syndrome; Kawasaki, T., *Allergy 16*:178 (1967); Kawasaki, T., *Shonica (Pediatrics) 26*:935 (1985)) were said to contain elevated TNF levels which were related to progress of the pathology (Yone *et al., supra*).

Other investigators have described mAbs specific for recombinant human TNF which had neutralizing activity *in vitro* (Liang, C-M. *et al. Biochem. Biophys. Res. Comm. 137*:847-854 (1986); Meager, A. *et al., Hybridoma* 6:305-311 (1987); Fendly *et al., Hybridoma* 6:359-369 (1987); Bringman, T S *et al., Hybridoma* 6:489-507 (1987); Hirai, M. *et al., J. Immunol. Meth. 96*:57-62 (1987); Moller, A. *et al. (Cytokine* 2:162-169 (1990)). Some of these mAbs were used to map epitopes of human TNF and develop enzyme immunoassays (Fendly *et al., supra*; Hirai *et al., supra*; Moller *et al., supra*) and to assist in the purification of recombinant TNF (Bringman *et al., supra*). However, these studies do not provide a basis for producing TNF neutralizing antibodies that can be used for *in vivo* diagnostic or therapeutic uses in humans, due to immunogenicity, lack of specificity and/or pharmaceutical suitability.

Neutralizing antisera or mAbs to TNF have been shown in mammals other than man to abrogate adverse physiological changes and prevent death after lethal challenge in experimental endotoxemia and bacteremia. This effect has been demonstrated, e.g., in rodent lethality assays and in primate pathology model systems (Mathison, J.C. *et al., J. Clin. Invest. 81*:1925-1937 (1988);

Beutler, B. *et al., Science 229*:869-871 (1985); Tracey, K. J. *et al., Nature 330*:662-664 (1987); Shimamoto, Y. *et al., Immunol. Lett. 17*:311-318 (1988); Silva, A. T. *et al., J. Infect. Dis. 162*:421-427 (1990); Opal, S. M. *et al., J. Infect. Dis. 161*:1148-1152 (1990); Hinshaw, L.B. *et al., Circ. Shock 30*:279-292 (1990)).

To date, experience with anti-TNF mAb therapy in humans has been limited but shows beneficial therapeutic results, *e.g.*, in arthritis and sepsis. *See, e.g.*, Elliot, M. J. *et al., Baillieres Clin. Rheumatol. 9*:633-52 (1995); Feldmann M, *et al., Ann. N. Y. Acad. Sci. USA 766*:272-8 (1995); van der Poll, T. *et al., Shock 3*:1-12 (1995); Wherry *et al., Crit. Care. Med. 21*:S436-40 (1993); Tracey K. J., *et al., Crit. Care Med. 21*:S415-22 (1993).

Mammalian development is dependent on both the proliferation and differentiation of cells as well as programmed cell death which occurs through apoptosis (Walker, *et al., Methods Achiev. Exp. Pathol. 13*:18 (1988). Apoptosis plays a critical role in the destruction of immune thymocytes that recognize self antigens. Failure of this normal elimination process may play a role in autoimmune diseases (Gammon *et al., Immunology Today 12*:193 (1991)).

Itoh *et al.* (*Cell 66*:233 (1991)) described a cell surface antigen, Fas/CD95 that mediates apoptosis and is involved in clonal deletion of T-cells. Fas is expressed in activated T-cells, B-cells, neutrophils and in thymus, liver, heart and lung and ovary in adult mice (Watanabe-Fukunaga *et al., J. Immunol. 148*:1274 (1992)) in addition to activated T-cells, B-cells, neutorophils. In experiments where a monoclonal Ab is cross-linked to Fas, apoptosis is induced (Yonehara *et al., J. Exp. Med. 169*:1747 (1989); Trauth *et al., Science 245*:301 (1989)). In addition, there is an example where binding of a monoclonal Ab to Fas is stimulatory to T-cells under certain conditions (Alderson *et al., J. Exp. Med. 178*:2231 (1993)).

Fas antigen is a cell surface protein of relative MW of 45 Kd. Both human and murine genes for Fas have been cloned by Watanabe-Fukunaga *et al.*, (*J. Immunol. 148*:1274 (1992)) and Itoh *et al.* (*Cell 66*:233 (1991)). The proteins encoded by these genes are both transmembrane proteins with structural homology to the Nerve Growth Factor/Tumor Necrosis Factor receptor superfamily, which includes two TNF receptors, the low affinity Nerve Growth Factor receptor and CD40, CD27, CD30, and OX40.

Recently the Fas ligand has been described (Suda *et al., Cell 75*:1169 (1993)). The amino acid sequence indicates that Fas ligand is a type II transmembrane protein belonging to the TNF family. Thus, the Fas ligand polypeptide comprises three main domains: a short intracellular domain at the

amino terminal end and a longer extracellular domain at the carboxy terminal end, connected by a hydrophobic transmembrane domain. Fas ligand is expressed in splenocytes and thymocytes, consistent with T-cell mediated cytotoxicity. The purified Fas ligand has a MW of 40 kD.

Recently, it has been demonstrated that Fas/Fas ligand interactions are required for apoptosis following the activation of T-cells (Ju *et al.*, *Nature 373*:444 (1995); Brunner *et al.*, *Nature 373*:441 (1995)). Activation of T-cells induces both proteins on the cell surface. Subsequent interaction between the ligand and receptor results in apoptosis of the cells. This supports the possible regulatory role for apoptosis induced by Fas/Fas ligand interaction during normal immune responses.

Accordingly, there is a need to provide cytokines similar to TNF that are involved in pathological conditions. Such novel cytokines may be used to make novel antibodies or other antagonists that bind these TNF-like cytokines for diagnosis and therapy of disorders related to TNF-like cytokines.

Summary of the Invention

In accordance with one embodiment of the present invention, there is provided a novel extracellular domain of a Neutrokine-a polypeptide, and a novel extracellular domain of a Neutrokine-aSV polypeptide, as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof.

In accordance with another embodiment of the present invention, there are provided isolated nucleic acid molecules encoding human Neutrokine-a or Neutrokine-aSV, including mRNAs, DNAs, cDNAs, genomic DNAs as well as analogs and biologically active and diagnostically or therapeutically useful fragments and derivatives thereof.

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a cytokine and an apparent splice variant thereof that are structurally similar to TNF and related cytokines and have similar biological effects and activities. This cytokine is named Neutrokine-a and the invention includes Neutrokine-a polypeptides having at least a portion of the amino acid sequence in Figures 1A and 1B (SEQ ID NO:2) or amino acid sequence encoded by the cDNA clone (HNEDU15) deposited in a bacterial host on October 22, 1996 assigned ATCC number 97768. The nucleotide sequence determined by sequencing the deposited Neutrokine-a clone, which is shown in Figures 1A and 1B (SEQ ID NO:1), contains an open reading frame encoding a complete polypeptide of 285 amino acid residues including an N-terminal

methionine, a predicted intracellular domain of about 46 amino acid residues, a predicted transmembrane domain of about 26 amino acids, a predicted extracellular domain of about 213 amino acids, and a deduced molecular weight for the complete protein of about 31 kDa. As for other type II transmembrane proteins, soluble forms of Neutrokine-α include all or a portion of the extracellular domain cleaved from the transmembrane domain and a polypeptide comprising the complete Neutrokine-α polypeptide lacking the transmembrane domain, i.e., the extracellular domain linked to the intracellular domain.

The apparent splice variant of Neutrokine-a is named Neutrokine-aSV and the invention includes Neutrokine-aSV polypeptides having at least a portion of the amino acid sequence in Figures 5A and 5B (SEQ ID NO:19) or amino acid sequence encoded by the cDNA clone HDPMC52 deposited on December 10, 1998 and assigned ATCC number 203518. The nucleotide sequence determined by sequencing the deposited Neutrokine-aSV clone, which is shown in Figures 5A and 5B (SEQ ID NO:18), contains an open reading frame encoding a complete polypeptide of 266 amino acid residues including an N-terminal methionine, a predicted intracellular domain of about 46 amino acid residues, a predicted transmembrane domain of about 26 amino acids, a predicted extracellular domain of about 194 amino acids, and a deduced molecular weight for the complete protein of about 29 kDa. As for other type II transmembrane proteins, soluble forms of Neutrokine-aSV include all or a portion of the extracellular domain cleaved from the transmembrane domain and a polypeptide comprising the complete Neutrokine-aSV polypeptide lacking the transmembrane domain, i.e., the extracellular domain linked to the intracellular domain.

Thus, one embodiment of the invention provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a full-length Neutrokine-a polypeptide having the complete amino acid sequence in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (b) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokine-a polypeptide having the amino acid sequence at positions 73 to 285 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the clone contained in the deposit having ATCC accession number 97768; (c) a nucleotide sequence encoding a fragment of the polypeptide of (b) having Neutrokine-a functional activity (e.g., biological acitivity); (d) a nucleotide sequence encoding a polypeptide comprising the Neutrokine-a intracellular domain (predicted to constitute amino

acid residues from about 1 to about 46 in Figures 1A and 1B (SEQ ID NO:2)) or as encoded by the clone contained in the deposit having ATCC accession number 97768; (e) a nucleotide sequence encoding a polypeptide comprising the Neutrokine-α transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (f) a nucleotide sequence encoding a soluble Neutrokine-α polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain; and (g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above.

Another embodiment of the invention provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a full-length Neutrokine-αSV polypeptide having the complete amino acid sequence in Figures 5A and 5B (SEQ ID NO:19) or as encoded by the cDNA clone contained in the ATCC Deposit deposited on December 10, 1998 as ATCC Number 203518; (b) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokine-αSV polypeptide having the amino acid sequence at positions 73 to 266 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA clone contained in ATCC 203518 deposited on December 10, 1998; (c) a nucleotide sequence encoding a polypeptide comprising the Neutrokine-αSV intracellular domain (predicted to constitute amino acid residues from about 1 to about 46 in Figures 5A and 5B (SEQ ID NO:19)) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (d) a nucleotide sequence encoding a polypeptide comprising the Neutrokine-αSV transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 5A and 5B (SEQ ID NO:19) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (e) a nucleotide sequence encoding a soluble Neutrokine-αSV polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain; and (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), or (e) above.

Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g) above. This

polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a Neutrokine-α or Neutrokine-αSV polypeptide having an amino acid sequence in (a), (b), (c), (d), (e) or (f) above. A further nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of a Neutrokine-α or Neutrokine-αSV polypeptide having an amino acid sequence which contains at least one amino acid addition, substitution, and/or deletion but not more than 50 amino acid additions, substitutions and/or deletions, even more preferably, not more than 40 amino acid additions, substitutions, and/or deletions, still more preferably, not more than 30 amino acid additions, substitutions, and/or deletions, and still even more preferably, not more than 20 amino acid additions, substitutions, and/or deletions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Neutrokine-α or Neutrokine-αSV polypeptide to have an amino acid sequence which contains not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 or 1-100, 1-50, 1-25, 1-20, 1-15, 1-10, or 1-5 amino acid additions, substitutions and/or deletions. Conservative substitutions are preferable.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of Neutrokine-α polypeptides or peptides by recombinant techniques.

In accordance with a further embodiment of the present invention, there is provided a process for producing such polypeptide by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a human Neutrokine-α or Neutrokine-αSV nucleic acid sequence, under conditions promoting expression of said polypeptide and subsequent recovery of said polypeptide.

The invention further provides an isolated Neutrokine-α polypeptide comprising an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length Neutrokine-α polypeptide having the complete amino acid sequence shown in Figures 1A and 1B (i.e., positions 1-285 of SEQ ID NO:2) or as encoded by the cDNA clone contained in the

deposit having ATCC accession number 97768; (b) the amino acid sequence of the full-length Neutrokine-a polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions 2 to 285 of SEQ ID NO:2); (c) a fragment of the polypeptide of (b) having Neutrokine-a functional activity (e.g., biological activity);; (d) the amino acid sequence of the predicted extracellular domain of the Neutrokine-a polypeptide having the amino acid sequence at positions 73 to 285 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (e) the amino acid sequence of the Neutrokine-a intracellular domain (predicted to constitute amino acid residues from about 1 to about 46 in Figures 1A and 1B (SEQ ID NO:2)) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (f) the amino acid sequence of the Neutrokine-a transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 1A and 1B (SEQ ID NO:2)) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (g) the amino acid sequence of the soluble Neutrokine-a polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain, wherein each of these domains is defined above; and (g) fragments of the polypeptide of (a), (b), (c), (d), (e), or (f). The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e) (f), or (g) above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above. Additional embodiments of the invention relates to a polypeptide which comprises the amino acid sequence of an epitope-bearing portion of a Neutrokine-a polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), or (g) above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Neutrokine-a polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

The invention further provides an isolated Neutrokine-aSV polypeptide comprising an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length Neutrokine-aSV polypeptide having

the complete amino acid sequence shown in Figures 5A and 5B (i.e., positions 1-266 of SEQ ID NO:19) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (b) the amino acid sequence of the full-length Neutrokine-aSV polypeptide having the complete amino acid sequence shown in SEQ ID NO:19 excepting the N-terminal methionine (i.e., positions 2 to 266 of SEQ ID NO:19); (c) the amino acid sequence of the predicted extracellular domain of the Neutrokine-aSV polypeptide having the amino acid sequence at positions 73 to 266 in Figures 5A and 5B (SEQ ID NO:19) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (d) the amino acid sequence of the Neutrokine-aSV intracellular domain (predicted to constitute amino acid residues from about 1 to about 46 in Figures 5A and 5B (SEQ ID NO:19)) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (e) the amino acid sequence of the Neutrokine-aSV transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 5A and 5B (SEQ ID NO:19)) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (f) the amino acid sequence of the soluble Neutrokine-aSV polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain, wherein each of these domains is defined above; and (g) fragments of the polypeptide of (a), (b), (c), (d), (e), or (f). The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e) (f), or (g) above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above. Additional embodiments of the invention relates to a polypeptide which comprises the amino acid sequence of an epitope-bearing portion of a Neutrokine-aSV polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), or (g) above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Neutrokine-aSV polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

An additional embodiment of the invention relates to a polypeptide which has the amino acid sequence of an epitope-bearing portion of a

Neutrokine-α or Neutrokine-αSV polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f) or (g) above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Neutrokine-α or Neutrokine-αSV polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention. In another embodiment, the invention provides an isolated antibody that binds specifically (i.e., uniquely) to a polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f) or (g), above.

The invention further provides methods for isolating antibodies that bind specifically (i.e., uniquely) to a Neutrokine-α or Neutrokine-αSV polypeptide having an amino acid sequence as described herein. Such antibodies are useful diagnostically or therapeutically as described below.

The invention also provides for pharmaceutical compositions comprising soluble Neutrokine-α and/or Neutrokine-αSV polypeptides, particularly human Neutrokine-α and/or Neutrokine-αSV polypeptides which may be employed, for instance, to treat tumor and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases, graft versus host disease, stimulate peripheral tolerance, destroy some transformed cell lines, mediate cell activation and proliferation, and to mediate immune regulation and inflammatory responses.

The invention further provides compositions comprising a Neutrokine-α or Neutrokine-αSV polynucleotide or a Neutrokine-α or Neutrokine-αSV polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In preferred embodiments, the compositions of the invention comprise a Neutrokine-α and/or Neutrokine-αSV polynucleotide for expression of a Neutrokine-α and/or Neutrokine-αSV polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of a Neutrokine-α or Neutrokine-αSV gene (e.g., enhancement of a normal B-cell function by expanding B-cell numbers).

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by Neutrokine-α and/or Neutrokine-αSV which involves contacting cells which express Neutrokine-α and/or Neutrokine-αSV with the candidate compound,

assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another embodiment, a method for identifying Neutrokine-α and/or Neutrokine-αSV receptors is provided, as well as a screening assay for agonists and antagonists using such receptors. This assay involves determining the effect a candidate compound has on Neutrokine-α and/or Neutrokine-αSV binding to the Neutrokine-α and/or Neutrokine-αSV receptor. In particular, the method involves contacting a Neutrokine-α and/or Neutrokine-αSV receptor with a Neutrokine-α and/or Neutrokine-αSV polypeptide of the invention and a candidate compound and determining whether Neutrokine-α and/or Neutrokine-αSV polypeptide binding to the Neutrokine-α and/or Neutrokine-αSV receptor is increased or decreased due to the presence of the candidate compound. The antagonists may be employed to prevent septic shock, inflammation, cerebral malaria, activation of the HIV virus, graft-host rejection, bone resorption, rheumatoid arthritis, cachexia (wasting or malnutrition), and immune system function.

The present inventors have discovered that Neutrokine-α is expressed not only in cells of monocytic lineage, but also in kidney, lung, peripheral leukocyte, bone marrow, T cell lymphoma, B cell lymphoma, activated T cells, stomach cancer, smooth muscle, macrophages, and cord blood tissue. The present inventors have further discovered that Neutrokine-αSV appears to be expressed highly only in primary dendritic cells. For a number of disorders of these tissues and cells, such as tumor and tumor metastasis, infection of bacteria, viruses and other parasites, immunodeficiencies, septic shock, inflammation, cerebral malaria, activation of the HIV virus, graft-host rejection, bone resorption, rheumatoid arthritis and cachexia (wasting or malnutrition, it is believed that significantly higher or lower levels of Neutrokine-α and/or Neutrokine-αSV gene expression can be detected in certain tissues (e.g., bone marrow) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" Neutrokine-α and/or Neutrokine-αSV gene expression level, i.e., the Neutrokine-α and/or Neutrokine-αSV expression level in tissue or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves: (a) assaying Neutrokine-α and/or Neutrokine-αSV gene expression level in cells or

body fluid of an individual; (b) comparing the Neutrokine-a and/or Neutrokine-aSV gene expression level with a standard Neutrokine-a and/or Neutrokine-aSV gene expression level, whereby an increase or decrease in the assayed Neutrokine-a and/or Neutrokine-aSV gene expression level compared to the standard expression level is indicative of a disorder.

An additional embodiment of the invention is related to a method for treating an individual in need of an increased or constitutive level of Neutrokine-a and/or Neutrokine-aSV activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated Neutrokine-a and/or Neutrokine-aSV polypeptide of the invention or an agonist thereof.

A still further embodiment of the invention is related to a method for treating an individual in need of a decreased level of Neutrokine-a and/or Neutrokine-aSV activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an Neutrokine-a and/or Neutrokine-aSV antagonist. Preferred antagonists for use in the present invention are Neutrokine-a-specific and/or Neutrokine-aSV-specific antibodies.

Brief Description of the Figures

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figures 1A and 1B shows the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of Neutrokine-a. Amino acids 1 to 46 represent the predicted intracellular domain, amino acids 47 to 72 the predicted transmembrane domain (the double-underlined sequence), and amino acids 73 to 285, the predicted extracellular domain (the remaining sequence). Potential asparagine-linked glycosylation sites are marked in Figures 1A and 1B with a bolded asparagine symbol (N) in the Neutrokine-a amino acid sequence and a bolded pound sign (#) above the first nucleotide encoding that asparagine residue in the Neutrokine-a nucleotide sequence. Potential N-linked glycosylation sequences are found at the following locations in the Neutrokine-a amino acid sequence: N-124 through Q-127 (N-124, S-125, S-126, Q-127) and N-242 through C-245 (N-242, N-243, S-244, C-245).

Regions of high identity between Neutrokine-a, Neutrokine-aSV, TNF-a, TNF-b, LT-b, and the closely related Fas Ligand (an aligment of these

sequences is presented in Figure 2) are underlined in Figures 1A and 1B. These regions are not limiting and are labeled as Conserved Domain (CD)-I, CD-II, CD-III, CD-IV, CD-V, CD-VI, CD-VII, CD-VIII, CD-IX, CD-X, and CD-XI in Figures 1A and 1B.

Figures 2A and 2B show the regions of identity between the amino acid sequences of Neutrokine-a (SEQ ID NO:2) and Neutrokine-aSV (SEQ ID NO:19), and TNF-a ("TNFalpha" in Figures 2A and 2B; GenBank No. Z15026; SEQ ID NO:3), TNF-β ("TNFbeta" in Figures 2A and 2B; GenBank No. Z15026; SEQ ID NO:4), Lymphotoxin-b ("LTbeta" in Figures 2A and 2B; GenBank No. L11016; SEQ ID NO:5), and FAS ligand ("FASL" in Figures 2A and 2B; GenBank No. U11821; SEQ ID NO:6), determined by the "MegAlign" routine which is part of the computer program called "DNA*STAR." Residues that match the consensus are shaded.

Figure 3 shows an analysis of the Neutrokine-a amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, as predicted for the amino acid sequence of SEQ ID NO:2 using the default parameters of the recited computer programs. In the "Antigenic Index - Jameson-Wolf" graph, the indicate location of the highly antigenic regions of Neutrokine-a i.e., regions from which epitope-bearing peptides of the invention may be obtained. Antigenic polypeptides include from about Phe-115 to about Leu-147, from about Ile-150 to about Tyr-163, from about Ser-171 to about Phe-194, from about Glu-223 to about Tyr-247, and from about Ser-271 to about Phe-278, of the amino acid sequence of SEQ ID NO:2.

The data presented in Figure 3 are also represented in tabular form in Table I. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 3, and Table I: "Res": amino acid residue of SEQ ID NO:2 and Figures 1A and 1B; "Position": position of the corresponding residue within SEQ ID NO:2 and Figures 1A and 1B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

Figures 4A, 4B, and 4C show the alignment of the Neutrokine-a nucleotide sequence determined from the human cDNA deposited in ATCC No. 97768 with related human cDNA clones of the invention which have been designated HSOAD55 (SEQ ID NO:7), HSLAH84 (SEQ ID NO:8) and HLTBM08 (SEQ ID NO:9).

Figures 5A and 5B shows the nucleotide (SEQ ID NO:18) and deduced amino acid (SEQ ID NO:19) sequences of the Neutrokine-aSV protein. Amino acids 1 to 46 represent the predicted intracellular domain, amino acids 47 to 72 the predicted transmembrane domain (the double-underlined sequence), and amino acids 73 to 266, the predicted extracellular domain (the remaining sequence). Potential asparagine-linked glycosylation sites are marked in Figures 5A and 5B with a bolded asparagine symbol (N) in the Neutrokine-aSV amino acid sequence and a bolded pound sign (#) above the first nucleotide encoding that asparagine residue in the Neutrokine-aSV nucleotide sequence. Potential N-linked glycosylation sequences are found at the following locations in the Neutrokine-aSV amino acid sequence: N-124 through Q-127 (N-124, S-125, S-126, Q-127) and N-223 through C-226 (N-223, N-224, S-225, C-226). Antigenic polypeptides include from about Pro-32 to about Leu-47, from about Glu-116 to about Ser-143, from about Phe-153 to about Tyr-173, from about Pro-218 to about Tyr-227, from about Ala-232 to about Gln-241; from about Ile-244 to about Ala-249; and from about Ser-252 to about Val-257 of the amino acid sequence of SEQ ID NO:19.

Regions of high identity between Neutrokine-a, Neutrokine-aSV, TNF-a, TNF-b, LT-b, and the closely related Fas Ligand (an aligment of these sequences is presented in Figure 2) are underlined in Figures 1A and 1B. Polypeptides comprising, or alternatively, consisting of the amino acid sequence of any combination of one, two, three, four, five, six, seven, eight, nine, ten, or or all of these regions are encompassed by the invention. These conserved regions (of Neutrokine-a and Neutrokine-aSV) are labeled as Conserved Domain (CD)-I, CD-II, CD-III, CD-V, CD-VI, CD-VII, CD-VIII, CD-IX, CD-X, and CD-XI in Figures 5A and 5B. Neutrokine-aSV does not contain the sequence of CD-IV described in the legend of Figures 1A and 1B.

An additional alignment of the Neutrokine-a polypeptide sequence (SEQ ID NO:2) with APRIL, TNF alpha, and LT alpha is presented in Figure 7A. In Figure 7A, beta sheet regions are indicated as described below in the Figure 7A legend.

Figure 6 shows an analysis of the Neutrokine-a amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity;

amphipathic regions; flexible regions; antigenic index and surface probability are shown, as predicted for the amino acid sequence of SEQ ID NO:2 using the default parameters of the recited computer programs. In the "Antigenic Index - Jameson-Wolf" graph, the indicate location of the highly antigenic regions of the Neutrokine-a protein, i.e., regions from which epitope-bearing peptides of the invention may be obtained. The data shown in Figure 6 can be easily represented in tabular format similar to the data shown in Table I. Such a tablular representation of the exact data disclosed in Figure 6 can be generated using the MegAlign component of the DNA*STAR computer sequence analysis package set on default parameters. This is the identical program that was used to generate Figures 3 and 6 of the present application.

Figure 7A. Sequence and expression of Neutrokine-a. Amino-acid sequence of Neutrokine-a and alignment with APRIL, TNF alpha, and LT alpha. The full length aa sequence of Neutrokine-a is shown together with an alignment of its predicted receptor-ligand binding domain with those of APRIL, TNF alpha, and LT alpha. The predicted membrane spanning region is indicated and the site of cleavage depicted with an arrow. Identical amino acids are shaded in yellow. Sequences overlaid with lines (A thru H) represent predicted beta-pleated sheet regions.

Figure 7B. Expression of Neutrokine-a mRNA. Northern hybridization analysis was performed using the Neutrokine-a orf as a probe on blots of poly(A)+ RNA (Clonetech) from a spectrum of human tissue types and a selection of cancer cell lines.

Figures 8A and 8B. Cell surface expression of Neutrokine-a on tumor cell lines and normal monocytes as detected by monoclonal antibody 12D6A. **A.** Single cell suspensions were prepared and cells resuspended at 10^7/mL. One million cells were stained with the anti-Neutrokine-a antibody or an isotype matched control mAb. Binding was detected by addition of PE-labeled goat anti mouse IgG. Cells were washed and analyzed on a Becton Dickinson FACScan using the CellQuest software provided by the manufacturer. **B.** Flow cytometric analysis of Neutrokine-a protein expression on *in vitro* cultured monocytes. Comparable results were obtained with monocytes from three different donors in three independent experiments.

Figures 9A and 9B. Neutrokine-a induced proliferation of human tonsillar B cells. **A.** The biological activity of Neutrokine-a was assessed in a standard B-lymphocyte co-stimulation assay utilizing either SAC or anti-IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as

measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolation of human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220). Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation is quantitated by a 20h pulse (1uCi/well) with ^{3}H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively. **B.** Neutrokine induced proliferation of anti-IgM primed B cells.

Figure 10. Biological assessment of amino-terminal truncations of Neutrokine-a. Using the standard B cell proliferation assay various truncations of Neutrokine-a were tested for biological activity.

Figure 11. Neutrokine-a binding to tonsillar B cells and the myeloma cell line IM9. Purified Neutrokine-a was labeled with biotin using the EZ-linkTM NHS-Biotin reagent (Pierce, Rockford, IL). The resultant protein was added to either normal or neoplastic cells. Following a shrot incubation, the labeled protein was wahsed out and PE-labeled strepavidin added to visualize Neutrokine-alpha binding. Cells were washed and analyzed on a Becton Dickinson FACScan using the CellQuest software provided by the manufacturer. Similar results were observed with a FLAG-tagged Neutrokine-a protein.

Figures 12A and 12B. *In vivo* administration of Neutrokine-a results in disruption of splenic architecture and appearance of a mature B cell population. **A.** Analyses of formalin-fixed spleens taken from normal (left) and Neutrokine-a treated (2mg/Kg, BID, 4d) mice (right). Upper panels are sections stained with H&E while lower figures have been staiend with a B cell marker, anti-CD45R(B220), and developed with peroxidase/DAB to visualize B cells. **B.** FACS analysis of B-cell population in spleen. CD45R (B220) and ThB (Ly 6D) expression on splenocytes from BALB/c mice injected with control buffer or (2mg/Kg, BID, 4d) Neutrokine-a protein.

Detailed Description

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a Neutrokine-a polypeptides having the

amino acid sequences shown in Figures 1A and 1B (SEQ ID NO:2), which was determined by sequencing a cDNA clone. The nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1) was obtained by sequencing the HNEDU15 clone, which was deposited on October 22, 1996 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and assigned ATCC Accession No. 97768. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding Neutrokine-aSV polypeptides having the amino acid sequences shown in Figures 5A and 5B (SEQ ID NO:19), which was determined by sequencing a cDNA clone. The nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18) was obtained by sequencing the HDPMC52 clone, which was deposited on December 10, 1998 at the American Type Culture Collection, and assigned ATCC Accession No. 203518. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

The Neutrokine-a and Neutrokine-a polypeptides of the present invention share sequence homology with the translation products of the human mRNAs for TNF-a, TNF-β, LTbeta, Fas ligand, APRIL, and LTalpha. (*See*, Figures 2A, 2B, and 7A). As noted above, TNF-a is thought to be an important cytokine that plays a role in cytotoxicity, necrosis, apoptosis, costimulation, proliferation, lymph node formation, immunoglobulin class switch, differentiation, antiviral activity, and regulation of adhesion molecules and other cytokines and growth factors.

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc., Foster City, CA), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art.

As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

Using the information provided herein, such as the nucleotide sequence in Figures 1A and 1B, a nucleic acid molecule of the present invention encoding a Neutrokine-α polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figures 1A and 1B (SEQ ID NO:1) was discovered in a cDNA library derived from neutrophils. Expressed sequence tags corresponding to a portion of the Neutrokine-α cDNA were also found in kidney, lung, peripheral leukocyte, bone marrow, T cell lymphoma, B cell lymphoma, activated T cells, stomach cancer, smooth muscle, macrophages, and cord blood tissue. In addition, using the nucleotide information provided in Figures 5A and 5B, a nucleic acid molecule of the present invention encoding a Neutrokine-αSV polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figures 5A and 5B (SEQ ID NO:18) was discovered in a cDNA library derived from primary dendritic cells.

The deposited clone contains an open reading frame encoding a protein of about 285 amino acid residues, a predicted intracellular domain of about 46 amino acids (amino acid residues from about 1 to about 46 in Figures 1A and 1B (SEQ ID NO:2)), a predicted transmembrane domain of about 26 amino acids (underlined amino acid residues from about 47 to about 72 in Figures 1A and 1B (SEQ ID NO:2)), a predicted extracellular domain of about 213 amino acids (amino acid residues from about 73 to about 285 in Figures 1A and 1B (SEQ ID NO:2)); and a deduced molecular weight of about 31 kDa. The Neutrokine-α polypeptide shown in Figures 1A and 1B (SEQ ID NO:2) is about

20% similar and about 10 % identical to human TNF-α which can be accessed on GenBank as Accession No. 339764.

The Neutrokine-aSV gene contains an open reading frame encoding a protein of about 266 amino acid residues, a predicted intracellular domain of about 46 amino acids (amino acid residues from about 1 to about 46 in Figures 5A and 5B (SEQ ID NO:19)), a predicted transmembrane domain of about 26 amino acids (underlined amino acid residues from about 47 to about 72 in Figures 5A and 5B (SEQ ID NO:19)), a predicted extracellular domain of about 194 amino acids (amino acid residues from about 73 to about 266 in Figures 5A and 5B (SEQ ID NO:19)); and a deduced molecular weight of about 29 kDa. The Neutrokine-aSV polypeptide shown in Figures 5A and 5B (SEQ ID NO:19) is about 33.9% similar and about 22.0% identical to human TNF-α which can be accessed on GenBank as Accession No. 339764.

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, the actual complete Neutrokine-a and/or Neutrokine-aSV polypeptides encoded by the deposited cDNAs, which comprise about 285 and 266 amino acids, respectively, may be somewhat shorter. In particular, the determined Neutrokine-a and Neutrokine-aSV coding sequences contain a common second methionine codon which may serve as an alternative start codon for translation of the open reading frame, at nucleotide positions 210-212 in Figures 1A and 1B (SEQ ID NO:1) and at nucleotide positions 64-66 in Figures 5A and 5B (SEQ ID NO:18). More generally, the actual open reading frame may be anywhere in the range of ±20 amino acids, more likely in the range of ±10 amino acids, of that predicted from either the first or second methionine codon from the N-terminus shown in Figures 1A and 1B (SEQ ID NO:1) and in Figures 5A and 5B (SEQ ID NO:18). It will further be appreciated that, the polypeptide domains described herein have been predicted by computer analysis, and accordingly, that depending on the analytical criteria used for identifying various functional domains, the exact "address" of the extracellular, intracellular and transmembrane domains of the Neutrokine-a and Neutrokine-aSV polypeptides may differ slightly. For example, the exact location of the Neutrokine-a and Neutrokine-aSV extracellular domains in Figures 1A and 1B (SEQ ID NO:2) and Figures 5A and 5B (SEQ ID NO:19) may vary slightly (e.g., the address may "shift" by about 1 to about 20 residues, more likely about 1 to about 5 residues) depending on the criteria used to define the domain. In this case, the ends of the transmembrane domains and the beginning of the extracellular domains were predicted on the basis of the identification of the hydrophobic amino acid sequence in the above

indicated positions, as shown in Figures 3 and 6 and in Table I. In any event, as discussed further below, the invention further provides polypeptides having various residues deleted from the N-terminus and/or C-terminus of the complete polypeptides, including polypeptides lacking one or more amino acids from the N-termini of the extracellular domains described herein, which constitute soluble forms of the extracellular domains of the Neutrokine-α and Neutrokine-αSV polypeptides.

As indicated, nucleic acid molecules and polynucleotides of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule (DNA or RNA), which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogeneous solution containing the clone and other members of the library) or a chromosome isolated or removed from a cell or a cell lysate (e.g., a "chromosome spread", as in a karyotype), is not "isolated" for the purposes of this invention. As discussed further herein, isolated nucleic acid molecules according to the present invention may be produced naturally, recombinantly, or synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) with an initiation codon at positions 147-149 of the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1). In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above, but which due to the degeneracy of the genetic code, still encode the Neutrokine-α protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate the

degenerate variants described above. In another embodiment, the invention provides isolated nucleic acid molecules encoding the Neutrokine-a polypeptide having an amino acid sequence encoded by the cDNA contained in the plasmid having ATCC accession number 97768. Preferably, this nucleic acid molecule comprises a sequence encoding the extracellular domain of the polypeptide encoded by the cDNA contained in the plasmid having ATCC accession number 97768.

Isolated nucleic acid molecules of the present invention also include DNA molecules comprising an open reading frame (ORF) with an initiation codon at positions 1-3 of the nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18). In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above, but which due to the degeneracy of the genetic code, still encode the Neutrokine-aSV polypeptide. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above. In another embodiment, the invention provides isolated nucleic acid molecules encoding the Neutrokine-aSV polypeptide having an amino acid encoded by the cDNA contained in the plasmid having ATCC accession number 203518. Preferably, this nucleic acid molecule comprises a sequence encoding the extracellular domain of the polypeptide encoded by the cDNA contained in the plasmid having ATCC accession number 203518.

The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1) or the nucleotide sequence of the Neutrokine-a cDNA contained in the plasmid having ATCC accession number 97768, or a nucleic acid molecule having a sequence complementary to one of the above sequences. In addition, the invention provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18) or the nucleotide sequence of the Neutrokine-a SV cDNA contained in the plasmid having ATCC accession number 203518, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, have uses which include, but are not limited to, as probes for gene mapping by *in situ* hybridization with chromosomes, and for detecting expression of the Neutrokine-a and Neutrokine-aSV in human tissue, for instance, by Northern or Western blot analysis.

The invention also provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 and SEQ ID NO:18

which have been determined from the following related cDNA clones: HSOAD55 (SEQ ID NO:7), HSLAH84 (SEQ ID NO:8), and HLTBM08 (SEQ ID NO:9).

The present invention is further directed to nucleic acid molecules encoding portions of the nucleotide sequences described herein, as well as to fragments of the isolated nucleic acid molecules described herein. In one embodiment, the invention provides a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:1 which consists of the nucleotides at positions 1-1001 of SEQ ID NO:1. In antoher embodiment, the invention provides a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:18 which consists of positions 1-798 of SEQ ID NO:18.

The present invention is further directed to fragments of the nucleic acid molecules (i.e. polynucleotides) described herein. By a fragment of a nucleic acid molecule having, for example, the nucleotide sequence of the cDNA contained in the plasmid having ATCC accession number 97768, a nucleotide sequence encoding the polypeptide sequence encoded by the cDNA contained in the plasmid having ATCC accession number 97768, the nucleotide sequence of SEQ ID NO:1, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2, the nucleotide sequence of the cDNA contained in the plasmid having ATCC accession number 203518, a nucleotide sequence encoding the polypeptide sequence encoded by the cDNA contained in the plasmid having ATCC accession number 203518, the nucleotide sequence of SEQ ID NO:18, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:20, or the complementary strand thereto, is intended fragments at least 15 nt, and more preferably at least 20 nt or at least 25nt, still more preferably at least 30 nt, and even more preferably, at least 40, 50, 100, 150, 200, 250, 300, 325, 350, 375, 400, 450, or 500 nt in length. These fragments have numerous uses which include, but are not limited to, diagnostic probes and primers as discussed herein. Of course, larger fragments, such as those of 501-1500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequences of the cDNA contained in the plasmid having ATCC accession number 97768, the nucleotide sequence of SEQ ID NO:1, the nucleotide sequences of the cDNA contained in the plasmid having ATCC accession number 203518, and the nucleotide sequence of SEQ ID NO:18. By a fragment at least 20 nt in length, for example, is intended fragments which include the particularly recited ranges of nucleotides from the nucleotide sequence of the deposited cDNAs or the nucleotide sequence as shown in Figures 1A and 1B (SEQ ID NO:1) or in Figures 5A and 5B (SEQ ID

NO:18), wherein the fragments may be larger or smaller than the particularly recited range by several (i.e. 5, 4, 3, 2 or 1) amino acids, at either extreme or at both extremes. Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the Neutrokine-α and/or Neutrokine-αSV polypeptide as identified in Figures 1A and 1B (SEQ ID NO:2) and in Figures 5A and 5B (SEQ ID NO:19), respectively, and described in more detail below.

Representative examples of Neutrokine-α polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to 50, 51 to 100, 101 to 146, 147 to 200, 201 to 250, 251 to 300, 301 to 350, 351 to 400, 401 to 450, 451 to 500, 501 to 550, 551 to 600, 600 to 650, 651 to 700, 701 to 750, 751 to 800, 800 to 850, 851 to 900, 901 to 950, 951 to 1000, 1001 to 1050, and/or 1051 to 1082, of SEQ ID NO:1, or the complementary strand thereto, or the cDNA contained in the plasmid having ATCC accession number 97768. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

Additional representative examples of Neutrokine-αSV polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to 50, 51 to 100, 101 to 150, 151 to 200, 201 to 250, 251 to 300, 301 to 350, 351 to 400, 401 to 450, 451 to 500, 501 to 550, 551 to 600, 600 to 650, 651 to 700, 701 to 750, 751 to 800, 800 to 850, and/or 851 to 900 of SEQ ID NO:18, or the complementary strand thereto, or the cDNA contained in the plasmid having ATCC accession number 203518. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

In certain preferred embodiments, polynucleotide of the invention comprise, or alternatively, consist of nucleotide residues 571-627, 580-627, 590-627, 600-627, 610-627, 571-620, 580-620, 590-620, 600-620, 571-610, 580-610, 590-610, 571-600, 580-600, and/or 571-590 of SEQ ID NO:1.

In certain other preferred embodiments, polynucleotide of the invention comprise, or alternatively, consist of nucleotide residues 1-879, 25-879, 50-879, 75-879, 100-879, 125-879, 150-879, 175-879, 200-879, 225-879, 250-879, 275-879, 300-879, 325-879, 350-879, 375-879, 400-879, 425-879, 450-879, 475-879, 500-879, 525-879, 550-879, 575-879, 600-879, 625-879, 650-879, 675-879, 700-879, 725-879, 750-879, 775-879, 800-879, 825-879,

850-879, 1-850, 25-850, 50-850, 75-850, 100-850, 125-850, 150-850, 175-850, 200-850, 225-850, 250-850, 275-850, 300-850, 325-850, 350-850, 375-850, 400-850, 425-850, 450-850, 475-850, 500-850, 525-850, 550-850, 575-850, 600-850, 625-850, 650-850, 675-850, 700-850, 725-850, 750-850, 775-850, 800-850, 825-850, 1-825, 25-825, 50-825, 75-825, 100-825, 125-825, 150-825, 175-825, 200-825, 225-825, 250-825, 275-825, 300-825, 325-825, 350-825, 375-825, 400-825, 425-825, 450-825, 475-825, 500-825, 525-825, 550-825, 575-825, 600-825, 625-825, 650-825, 675-825, 700-825, 725-825, 750-825, 775-825, 800-825, 1-800, 25-800, 50-800, 75-800, 100-800, 125-800, 150-800, 175-800, 200-800, 225-800, 250-800, 275-800, 300-800, 325-800, 350-800, 375-800, 400-800, 425-800, 450-800, 475-800, 500-800, 525-800, 550-800, 575-800, 600-800, 625-800, 650-800, 675-800, 700-800, 725-800, 750-800, 775-800, 1-775, 25-775, 50-775, 75-775, 100-775, 125-775, 150-775, 175-775, 200-775, 225-775, 250-775, 275-775, 300-775, 325-775, 350-775, 375-775, 400-775, 425-775, 450-775, 475-775, 500-775, 525-775, 550-775, 575-775, 600-775, 625-775, 650-775, 675-775, 700-775, 725-775, 750-775, 1-750, 25-750, 50-750, 75-750, 100-750, 125-750, 150-750, 175-750, 200-750, 225-750, 250-750, 275-750, 300-750, 325-750, 350-750, 375-750, 400-750, 425-750, 450-750, 475-750, 500-750, 525-750, 550-750, 575-750, 600-750, 625-750, 650-750, 675-750, 700-750, 725-750, 1-725, 25-725, 50-725, 75-725, 100-725, 125-725, 150-725, 175-725, 200-725, 225-725, 250-725, 275-725, 300-725, 325-725, 350-725, 375-725, 400-725, 425-725, 450-725, 475-725, 500-725, 525-725, 550-725, 575-725, 600-725, 625-725, 650-725, 675-725, 700-725, 1-700, 25-700, 50-700, 75-700, 100-700, 125-700, 150-700, 175-700, 200-700, 225-700, 250-700, 275-700, 300-700, 325-700, 350-700, 375-700, 400-700, 425-700, 450-700, 475-700, 500-700, 525-700, 550-700, 575-700, 600-700, 625-700, 650-700, 675-700, 1-675, 25-675, 50-675, 75-675, 100-675, 125-675, 150-675, 175-675, 200-675, 225-675, 250-675, 275-675, 300-675, 325-675, 350-675, 375-675, 400-675, 425-675, 450-675, 475-675, 500-675, 525-675, 550-675, 575-675, 600-675, 625-675, 650-675, 1-650, 25-650, 50-650, 75-650, 100-650, 125-650, 150-650, 175-650, 200-650, 225-650, 250-650, 275-650, 300-650, 325-650, 350-650, 375-650, 400-650, 425-650, 450-650, 475-650, 500-650, 525-650, 550-650, 575-650, 600-650, 625-650, 1-625, 25-625, 50-625, 75-625, 100-625, 125-625, 150-625, 175-625, 200-625, 225-625, 250-625, 275-625, 300-625, 325-625, 350-625, 375-625, 400-625, 425-625, 450-625, 475-625, 500-625, 525-625, 550-625, 575-625, 600-625, 1-600, 25-600, 50-600, 75-600, 100-600, 125-600, 150-600, 175-600, 200-600, 225-600, 250-600, 275-600, 300-600, 325-600, 350-600, 375-600,

400-600, 425-600, 450-600, 475-600, 500-600, 525-600, 550-600, 575-600, 1-575, 25-575, 50-575, 75-575, 100-575, 125-575, 150-575, 175-575, 200-575, 225-575, 250-575, 275-575, 300-575, 325-575, 350-575, 375-575, 400-575, 425-575, 450-575, 475-575, 500-575, 525-575, 550-575, 1-550, 25-550, 50-550, 75-550, 100-550, 125-550, 150-550, 175-550, 200-550, 225-550, 250-550, 275-550, 300-550, 325-550, 350-550, 375-550, 400-550, 425-550, 450-550, 475-550, 500-550, 525-550, 1-525, 25-525, 50-525, 75-525, 100-525, 125-525, 150-525, 175-525, 200-525, 225-525, 250-525, 275-525, 300-525, 325-525, 350-525, 375-525, 400-525, 425-525, 450-525, 475-525, 500-525, 1-500, 25-500, 50-500, 75-500, 100-500, 125-500, 150-500, 175-500, 200-500, 225-500, 250-500, 275-500, 300-500, 325-500, 350-500, 375-500, 400-500, 425-500, 450-500, 475-500, 1-475, 25-475, 50-475, 75-475, 100-475, 125-475, 150-475, 175-475, 200-475, 225-475, 250-475, 275-475, 300-475, 325-475, 350-475, 375-475, 400-475, 425-475, 450-475, 1-450, 25-450, 50-450, 75-450, 100-450, 125-450, 150-450, 175-450, 200-450, 225-450, 250-450, 275-450, 300-450, 325-450, 350-450, 375-450, 400-450, 425-450, 1-425, 25-425, 50-425, 75-425, 100-425, 125-425, 150-425, 175-425, 200-425, 225-425, 250-425, 275-425, 300-425, 325-425, 350-425, 375-425, 400-425, 1-400, 25-400, 50-400, 75-400, 100-400, 125-400, 150-400, 175-400, 200-400, 225-400, 250-400, 275-400, 300-400, 325-400, 350-400, 375-400, 1-375, 25-375, 50-375, 75-375, 100-375, 125-375, 150-375, 175-375, 200-375, 225-375, 250-375, 275-375, 300-375, 325-375, 350-375, 1-350, 25-350, 50-350, 75-350, 100-350, 125-350, 150-350, 175-350, 200-350, 225-350, 250-350, 275-350, 300-350, 325-350, 1-325, 25-325, 50-325, 75-325, 100-325, 125-325, 150-325, 175-325, 200-325, 225-325, 250-325, 275-325, 300-325, 1-300, 25-300, 50-300, 75-300, 100-300, 125-300, 150-300, 175-300, 200-300, 225-300, 250-300, 275-300, 1-275, 25-275, 50-275, 75-275, 100-275, 125-275, 150-275, 175-275, 200-275, 225-275, 250-275, 1-250, 25-250, 50-250, 75-250, 100-250, 125-250, 150-250, 175-250, 200-250, 225-250, 1-225, 25-225, 50-225, 75-225, 100-225, 125-225, 150-225, 175-225, 200-225, 1-200, 25-200, 50-200, 75-200, 100-200, 125-200, 150-200, 175-200, 1-175, 25-175, 50-175, 75-175, 100-175, 125-175, 150-175, 1-150, 25-150, 50-150, 75-150, 100-150, 125-150, 1-125, 25-125, 50-125, 75-125, 100-125, 1-100, 25-100, 50-100, 75-100, 1-75, 25-75, 50-75, 1-50, 25-50, and 1-25 of SEQ ID NO:18.

In certain additional preferred embodiments, polynucleotide of the invention comprise, or alternatively, consist of nucleotide residues 400-627, 425-627, 450-627, 475-627, 500-627, 525-627, 550-627, 575-627, 600-627,

400-600, 425-600, 450-600, 475-600, 500-600, 525-600, 550-600, 575-600, 400-575, 425-575, 450-575, 475-575, 500-575, 525-575, 550-575, 400-550, 425-550, 450-550, 475-550, 500-550, 525-550, 400-500, 425-500, 450-500, 475-500, 400-475, 425-475, 450-475, 400-450, 425-450, 571-800, 600-800, 625-800, 650-800, 675-800, 700-800, 725-800, 750-800, 775-800, 571-775, 600-775, 625-775, 650-775, 675-775, 700-775, 725-775, 750-775, 571-750, 600-750, 625-750, 650-750, 675-750, 700-750, 725-750, 571-725, 600-725, 625-725, 650-725, 675-725, 700-725, 571-700, 600-700, 625-700, 650-700, 675-700, 571-675, 600-675, 625-675, 650-675, 571-650, 600-650, 625-650, 571-625, 600-625, and/or 571-600 of SEQ ID NO:1.

In additional preferred embodiments, polynucleotide of the invention comprise, or alternatively, consist of nucleotide residues 147-500, 147-450, 147-400, 147-350, 200-500, 200-450, 200-400, 200-350, 250-500, 250-450, 250-400, 250-350, 300-500, 300-450, 300-400, 300-350, 350-750, 350-700, 350-650, 350-600, 350-550, 400-750, 400-700, 400-650, 400-600, 400-550, 425-750, 425-700, 425-650, 425-600, 425-550, 450-1020, 450-1001, 450-950, 450-900, 450-850, 450-800, 450-775, 500-1001, 500-950, 500-900, 500-850, 500-800, 500-775, 550-1001, 550-950, 550-900, 550-850, 550-800, 550-775, 600-1001, 600-950, 600-900, 600-850, 600-800, 600-775, 650-1001, 650-950, 650-900, 650-850, 650-800, 650-775, 700-1001, 700-950, 700-900, 700-850, 700-800, 700-775, 825-1082, 850-1082, 875-1082, 900-1082, 925-1082, 950-1082, 975-1082, 1000-1082, 1025-1082, and/or 1050-1082 of SEQ ID NO:1.

In additional specific embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain (amino acids 1 to 46 of SEQ ID NO:2), the predicted transmembrane domain (amino acids 47 to 72 of SEQ ID NO:2), the predicted extracellular domain (amino acids 73 to 285 of SEQ ID NO:2), or the predicted TNF conserved domain (amino acids 191 to 284 of SEQ ID NO:2) of Neutrokine-α. In additional embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of any combination of 1, 2, 3, or all 4 of the above recited domains.

In additional specific embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain (amino acids 1 to 46 of SEQ ID NO:19), the predicted transmembrane domain (amino acids 47 to 72 of SEQ ID NO:19), the predicted extracellular domain (amino acids 73 to 266 of SEQ ID NO:19), or the predicted TNF conserved domain (amino acids 172 to 265 of SEQ ID NO:19)

of Neutrokine-α. In additional embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of any combination of 1, 2, 3, or all 4 of the above recited domains.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a Neutrokine-α and/or Neutrokine-αSV functional activity. By a polypeptide demonstrating "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length *and/or* secreted Neutrokine-α polypeptide and/or Neutrokine-αSV polypeptide. Such functional activities include, but are not limited to, biological activity (e.g., ability to stimulate B cell proliferation, differentiation, and/or activation), antigenicity [ability to bind (or compete with a Neutrokine-α and/or Neutrokine-αSV polypeptide for binding) to an anti-Neutrokine-α and/or anti-Neutrokine-αSV antibody], immunogenicity (ability to generate antibody which binds to a Neutrokine-α and/or Neutrokine-αSV polypeptide), ability to form multimers with Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention, and ability to bind to a receptor or ligand for a Neutrokine-α and/or Neutrokine-αSV polypeptide (e.g., DRS (*See*, International Publication No. WO 98/41629), TR10 (*See*, International Publication No. WO 98/54202), 312C2 (*See*, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (*See*, U.S. Application Serial No. 09/176,200)).

The functional activity of Neutrokine-α and/or Neutrokine-αSV polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length Neutrokine-α and/or Neutrokine-αSV polypeptide for binding to anti- Neutrokine-α and/or anti-Neutrokine-αSV antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots,

precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a Neutrokine-α and/or Neutrokine-αSV ligand is identified (e.g., DR5 (*See*, International Publication No. WO 98/41629), TR10 (*See*, International Publication No. WO 98/54202), 312C2 (*See*, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (*See*, U.S. Application Serial No. 09/176,200)), or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducting gel chromotography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of Neutrokine-α and/or Neutrokine-αSV binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see, at least, Example 6) and otherwise known in the art may routinely be applied to measure the ability of Neutrokine-α and/or Neutrokine-αSV polypeptides and fragments, variants derivatives and analogs thereof to elicit Neutrokine-α and/or Neutrokine-αSV related biological activity (e.g., to stimulate, or alternatively to inhibit (in the case of Neutrokine-α and/or Neutrokine-αSV antagonists) B cell proliferation, differentiation and/or activation *in vitro or in vivo*).

Other methods will be known to the skilled artisan and are within the scope of the invention.

In additional embodiments, the polynucleotides of the invention encode functional attributes of Neutrokine-α and Neutrokine-αSV. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic

regions, flexible regions, surface-forming regions and high antigenic index regions of Neutrokine-a and Neutrokine-aSV polypeptides.

It is believed one or more of the beta pleated sheet regions of Neutrokine-α disclosed in Figure 7A is important for dimerization and also for interactions between Neutrokine-α and its ligands (e.g., Neutrokine-α polypeptides, and DR5 (*See*, International Publication No. WO 98/41629), TR10 (*See*, International Publication No. WO 98/54202), 312C2 (*See*, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (*See*, U.S. Application Serial No. 09/176,200)). Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of beta pleated sheet region A, A', B, B', C, D, E, F, G, or H disclosed in Figure 7A and described in Example 6. Additional embodiments of the invention are directed to polynucleotides encoding Neutrokine-α polypeptides which comprise, or alternatively consist of, any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or all 10 of beta pleated sheet regions A-H disclosed in Figure 7A and described in Example 6. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the Neutrokine-α amino acid sequence of beta pleated sheet region A, A', B, B', C, D, E, F, G, or H disclosed in Figure 7A and described in Example 6. Additional embodiments of the invention are directed Neutrokine-α polypeptides which comprise, or alternatively consist of, any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or all 10 of beta pleated sheet regions A-H disclosed in Figure 7A and described in Example 6.

Certain preferred regions in this regard are set out in Figure 3 (Table I). The data presented in Figure 3 and that presented in Table I, merely present a different format of the same results obtained when the amino acid sequence of SEQ ID NO:2 is analyzed using the default parameters of the DNA*STAR computer algorithm.

The above-mentioned preferred regions set out in Figure 3 and in Table I include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in Figures 1A and 1B. As set out in Figure 3 and in Table I, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and coil-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic

regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides in this regard are those that encode polypeptides comprising regions of Neutrokine-a and/or Neutrokine-aSV that combine several structural features, such as several (e.g., 1, 2, 3 , or 4) of the features set out above.

Additionally, the data presented in columns VIII, IX, XIII, and XIV of Table I can routinely be used to determine regions of Neutrokine-a which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response. The data presented in Figure 6 can also routinely be presented in a similar tabular format by simply examining the amino acid sequence disclosed in Figure 6 (SEQ ID NO:19) using the modules and algorithms of the DNA*STAR set on default parameters. As above, the amino acid sequence presented in Figure 6 can also be used to determine regions of Neutrokine-a which exhibit a high degree of potential for antigenicity whether presented as a Figure (as in Figure 6) or a table (as in Table 1).

Table 1

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	A	.	.	.	.	.	.	0.73	-0.71	.	.	.	0.95	1.39
Asp	2	A	.	.	.	.	T	.	1.12	-0.66	*	.	.	1.15	1.56
Asp	3	A	.	.	.	.	T	.	1.62	-1.09	*	.	.	1.15	2.12
Ser	4	A	.	.	.	.	T	.	2.01	-1.51	.	.	.	1.15	4.19
Thr	5	A	.	.	.	.	T	.	2.40	-2.13	.	.	F	1.30	4.35
Glu	6	A	A	.	.	.	.	.	2.70	-1.73	*	*	F	0.90	4.51
Arg	7	A	A	.	.	.	.	.	2.81	-1.34	*	*	F	0.90	4.51
Glu	8	A	A	.	.	.	.	.	2.00	-1.73	*	*	F	0.90	6.12
Gln	9	A	A	.	.	.	.	.	1.99	-1.53	*	*	F	0.90	2.91
Ser	10	A	.	.	B	.	.	.	2.00	-1.04	*	*	F	0.90	2.15
Arg	11	A	.	.	B	.	.	.	1.33	-0.66	*	*	F	0.90	1.66
Leu	12	A	.	.	B	.	.	.	0.41	-0.09	*	*	F	0.45	0.51
Thr	13	A	.	.	B	.	.	.	0.46	0.20	*	*	F	-0.15	0.32
Ser	14	A	A	.	.	.	.	.	0.50	-0.19	*	*	.	0.30	0.32
Cys	15	A	A	.	.	.	.	.	0.91	-0.19	*	*		0.30	0.78
Leu	16	A	A	.	.	.	.	.	0.80	-0.87	*	*	F	0.90	1.06
Lys	17	A	A	.	.	.	.	.	1.61	-1.36	.	*	F	0.90	1.37
Lys	18	A	A	.	.	.	.	.	1.32	-1.74	.	*	F	0.90	4.44
Arg	19	A	A	.	.	.	.	.	1.67	-1.70	.	*	F	0.90	5.33
Glu	20	A	A	.	.	.	.	.	1.52	-2.39	.	*	F	0.90	5.33
Glu	21	A	A	.	.	.	.	.	2.38	-1.70	.	*	F	0.90	2.20
Met	22	A	A	.	.	.	.	.	2.33	-1.70	.	*	F	0.90	2.24
Lys	23	A	A	.	.	.	.	.	1.62	-1.70	*	*	F	0.90	2.24
Leu	24	A	A	.	.	.	.	.	0.66	-1.13	*	*	F	0.75	0.69
Lys	25	A	A	.	.	.	.	.	0.36	-0.49	.	*	F	0.45	0.52
Glu	26	A	A	.	B	.	.	.	-0.53	-0.71	*	*	.	0.60	0.35
Cys	27	A	A	.	B	.	.	.	-0.74	-0.03	*	*	.	0.30	0.30
Val	28	A	A	.	B	.	.	.	-1.00	-0.03	*	*	.	0.30	0.12
Ser	29	A	A	.	B	.	.	.	-0.08	0.40	*	*	.	-0.30	0.11
Ile	30	A	.	.	B	.	.	.	-0.08	0.40	*	*	.	-0.30	0.40
Leu	31	A	.	.	B	.	.	.	-0.08	-0.17	*	.	.	0.45	1.08
Pro	32	.	.	.	B	.	.	C	0.29	-0.81	*	.	F	1.10	1.39
Arg	33	.	.	.	.	T	.	.	0.93	-0.81	.	*	F	1.50	2.66
Lys	34	.	.	.	.	T	.	.	0.93	-1.07	.	.	F	1.84	4.98
Glu	35	.	.	.	.	.	.	C	0.97	-1.37	*	*	F	1.98	4.32
Ser	36	.	.	.	.	.	T	C	1.89	-1.16	*	*	F	2.52	1.64
Pro	37	.	.	.	.	.	T	C	1.80	-1.16	*	*	F	2.86	1.60
Ser	38	.	.	.	.	T	T	.	1.39	-0.77	*	.	F	3.40	1.24
Val	39	A	.	.	.	.	T	.	1.39	-0.39	.	*	F	2.36	1.24
Arg	40	A	.	.	.	.	.	.	1.39	-0.77	*	*	F	2.46	1.60
Ser	41	A	.	.	.	.	.	.	1.34	-1.20	*	*	F	2.46	2.00
Ser	42	.	.	.	.	T	T	.	1.60	-1.16	.	*	F	3.06	2.67
Lys	43	.	.	.	.	T	T	.	1.09	-1.80	.	*	F	3.06	2.72
Asp	44	.	.	.	.	T	T	.	1.13	-1.11	*	*	F	3.40	1.67
Gly	45	A	.	.	.	.	T	.	0.43	-0.81	*	*	F	2.66	1.03
Lys	46	A	A	.	.	.	.	.	0.14	-0.70	.	.	F	1.77	0.52
Leu	47	A	A	.	.	.	.	.	0.13	-0.20	*	.	.	0.98	0.31
Leu	48	A	A	.	.	.	.	.	-0.72	0.29	*	.	.	0.04	0.46
Ala	49	A	A	.	.	.	.	.	-1.53	0.54	.	*	.	-0.60	0.19
Ala	50	A	A	.	.	.	.	.	-2.00	1.23	.	.	.	-0.60	0.19
Thr	51	A	A	.	.	.	.	.	-2.63	1.23	.	.	.	-0.60	0.19
Leu	52	A	A	.	.	.	.	.	-2.63	1.04	.	.	.	-0.60	0.19
Leu	53	A	A	.	.	.	.	.	-2.63	1.23	.	.	.	-0.60	0.15
Leu	54	A	A	.	.	.	.	.	-2.34	1.41	.	.	.	-0.60	0.09
Ala	55	A	A	.	.	.	.	.	-2.42	1.31	.	.	.	-0.60	0.14
Leu	56	A	A	.	.	.	.	.	-2.78	1.20	.	.	.	-0.60	0.09
Leu	57	A	.	.	.	.	T	.	-2.78	1.09	.	.	.	-0.20	0.06
Ser	58	A	.	.	.	.	T	.	-2.28	1.09	.	.	.	-0.20	0.05
Cys	59	A	.	.	.	.	T	.	-2.32	1.07	.	.	.	-0.20	0.09

Table I (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Cys	60	A	.	.	.	.	T	.	-2.59	1.03	.	.	.	-0.20	0.08
Leu	61	.	.	B	B	.	.	.	-2.08	0.99	.	.	.	-0.60	0.04
Thr	62	.	.	B	B	.	.	.	-1.97	0.99	.	.	.	-0.60	0.11
Val	63	.	.	B	B	.	.	.	-1.93	1.20	.	.	.	-0.60	0.17
Val	64	.	.	B	B	.	.	.	-1.24	1.39	.	.	.	-0.60	0.33
Ser	65	.	.	B	B	.	.	.	-1.43	1.10	.	.	.	-0.60	0.40
Phe	66	A	.	.	B	.	.	.	-1.21	1.26	.	.	.	-0.60	0.40
Tyr	67	A	.	.	B	.	.	.	-1.49	1.11	.	.	.	-0.60	0.54
Gln	68	A	.	.	B	.	.	.	-1.44	0.97	.	.	.	-0.60	0.41
Val	69	A	.	.	B	.	.	.	-0.59	1.27	.	.	.	-0.60	0.39
Ala	70	A	.	.	B	.	.	.	-0.63	0.89	.	.	.	-0.60	0.43
Ala	71	A	.	.	B	.	.	.	0.07	0.56	.	*	.	-0.60	0.25
Leu	72	A	.	.	.	.	T	.	-0.50	0.16	.	*	.	0.10	0.55
Gln	73	A	.	.	.	.	T	.	-1.09	0.20	.	.	F	0.25	0.45
Gly	74	A	.	.	.	.	T	.	-0.53	0.20	.	.	F	0.25	0.45
Asp	75	A	.	.	.	.	T	.	-0.76	0.09	.	*	F	0.25	0.73
Leu	76	A	A	.	.	.	.	.	-0.06	0.09	.	*	F	-0.15	0.35
Ala	77	A	A	.	.	.	.	.	0.17	-0.31	.	*	.	0.30	0.69
Ser	78	A	A	.	.	.	.	.	0.17	-0.24	.	*	.	0.30	0.42
Leu	79	A	A	.	.	.	.	.	-0.30	-0.24	.	*	.	0.30	0.88
Arg	80	A	A	.	.	.	.	.	-0.30	-0.24	.	*	.	0.30	0.72
Ala	81	A	A	.	.	.	.	.	0.17	-0.34	.	*	.	0.30	0.93
Glu	82	A	A	.	.	.	.	.	0.72	-0.30	.	*	.	0.45	1.11
Leu	83	A	A	.	.	.	.	.	0.99	-0.49	.	*	.	0.30	0.77
Gln	84	A	A	.	.	.	.	.	1.21	0.01	.	*	.	-0.15	1.04
Gly	85	A	A	.	.	.	.	.	1.10	0.01	*	*	.	-0.30	0.61
His	86	A	A	.	.	.	.	.	1.73	0.01	*	*	.	-0.15	1.27
His	87	A	A	.	.	.	.	.	0.92	-0.67	.	*	.	0.75	1.47
Ala	88	A	A	.	.	.	.	.	1.52	-0.39	.	*	.	0.45	1.22
Glu	89	A	A	.	.	.	.	.	0.93	-0.39	.	.	.	0.45	1.39
Lys	90	A	A	.	.	.	.	.	0.93	-0.39	*	.	F	0.60	1.03
Leu	91	A	.	.	.	.	T	.	0.38	-0.46	*	.	.	0.85	1.01
Pro	92	A	.	.	.	.	T	.	0.07	-0.46	.	.	.	0.70	0.59
Ala	93	A	.	.	.	.	T	.	0.07	-0.03	.	.	.	0.70	0.29
Gly	94	A	.	.	.	.	T	.	-0.14	0.47	.	.	.	-0.20	0.36
Ala	95	A	.	.	.	.	.	.	-0.14	0.21	.	*	.	-0.10	0.36
Gly	96	A	.	.	.	.	.	.	0.08	-0.21	.	.	F	0.65	0.71
Ala	97	A	.	.	.	.	.	.	-0.06	-0.21	.	.	F	0.65	0.72
Pro	98	A	.	.	.	.	.	.	-0.28	-0.21	.	*	F	0.65	0.71
Lys	99	A	A	.	.	.	.	.	0.07	-0.03	.	.	F	0.45	0.59
Ala	100	A	A	.	.	.	.	.	0.66	-0.46	.	.	F	0.60	1.01
Gly	101	A	A	.	.	.	.	.	0.41	-0.96	.	.	F	0.90	1.13
Leu	102	A	A	.	.	.	.	.	0.79	-0.89	.	.	F	0.75	0.57
Glu	103	A	A	.	.	.	.	.	0.41	-0.46	*	.	F	0.45	0.88
Gln	104	A	A	.	.	.	.	.	-0.49	-0.46	*	.	F	0.45	0.89
Ala	105	A	A	.	.	.	.	.	-0.21	-0.24	.	.	.	0.30	0.81
Pro	106	A	A	.	.	.	.	.	-0.46	-0.44	.	.	.	0.30	0.67
Ala	107	A	A	.	.	.	.	.	0.01	0.06	.	.	.	-0.30	0.39
Val	108	A	A	.	.	.	.	.	-0.80	0.49	.	*	.	-0.60	0.38
Thr	109	A	A	.	.	.	.	.	-0.76	0.67	.	*	.	-0.60	0.20
Ala	110	A	A	.	.	.	.	.	-1.06	0.24	*	*	.	-0.30	0.40
Gly	111	A	A	.	.	.	.	.	-1.54	0.43	*	*	.	-0.60	0.38
Leu	112	A	A	.	.	.	.	.	-0.96	0.57	*	*	.	-0.60	0.23
Lys	113	.	A	B	.	.	.	.	-0.31	0.09	*	*	.	-0.30	0.39
Ile	114	.	A	B	.	.	.	.	-0.21	0.01	*	.	.	-0.30	0.61
Phe	115	.	A	B	.	.	.	.	-0.21	0.01	*	.	.	0.15	1.15
Glu	116	.	A	.	.	.	.	C	-0.08	-0.17	*	.	F	1.25	0.58
Pro	117	.	A	.	.	.	.	C	0.39	0.26	*	*	F	1.10	1.28
Pro	118	.	.	.	.	.	.	C	0.34	-0.00	.	.	F	2.20	1.47

Table I (continued)

Res	Position		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
	Ala	119	.	.	.	.	.	T	C	0.89	-0.79	.	*	F	3.00	1.47
	Pro	120	.	.	.	.	.	T	C	1.59	-0.36	.	*	F	2.25	0.94
	Gly	121	.	.	.	.	T	T	.	1.29	-0.39	.	*	F	2.15	0.98
	Gln	122	.	.	.	.	T	T	.	1.20	-0.43	.	.	F	2.00	1.30
	Gly	123	.	.	.	.	.	.	C	1.41	-0.54	.	.	F	1.60	1.12
	Asn	124	.	.	.	.	.	T	C	2.00	-0.37	.	.	F	1.50	1.97
	Ser	125	.	.	.	.	.	T	C	1.91	-0.60	.	*	F	1.50	1.82
	Ser	126	.	.	.	.	.	T	C	2.37	-0.21	.	*	F	1.54	2.47
	Gln	127	.	.	.	.	.	T	C	2.37	-0.64	.	*	F	2.18	3.01
	Asn	128	.	.	.	.	.	.	C	2.76	-0.64	.	.	F	2.32	3.61
	Ser	129	.	.	.	.	.	T	C	2.87	-1.03	.	.	F	2.86	5.39
	Arg	130	.	.	.	.	T	T	.	2.58	-1.41	*	.	F	3.40	6.09
	Asn	131	.	.	.	.	T	T	.	2.02	-1.31	*	.	F	3.06	3.83
	Lys	132	.	.	.	.	T	T	.	2.02	-1.07	*	.	F	2.72	2.12
	Arg	133	.	.	.	.	T	.	.	1.68	-1.06	*	.	F	2.18	1.88
	Ala	134	.	.	.	.	.	.	C	1.77	-0.63	*	.	F	1.64	1.15
	Val	135	.	.	.	.	.	.	C	1.66	-0.60	*	.	F	1.49	0.89
	Gln	136	.	.	.	.	.	.	C	1.66	-0.60	*	.	F	1.83	0.79
	Gly	137	.	.	.	.	.	T	C	1.30	-0.60	*	.	F	2.52	1.35
	Pro	138	.	.	.	.	.	T	C	0.33	-0.61	*	.	F	2.86	2.63
	Glu	139	.	.	.	.	T	T	.	0.61	-0.61	*	.	F	3.40	1.13
	Glu	140	A	.	.	.	.	T	.	1.47	-0.53	*	.	F	2.66	1.64
	Thr	141	A	.	.	.	.	.	.	1.47	-0.56	.	.	F	2.12	1.84
	Val	142	A	.	.	.	.	.	.	1.14	-0.99	.	.	F	1.78	1.77
	Thr	143	A	.	.	.	.	T	.	0.54	-0.41	.	.	F	1.19	0.55
	Gln	144	A	.	.	.	.	T	.	0.54	0.27	*	.	F	0.25	0.31
	Asp	145	A	.	.	.	.	T	.	-0.27	0.19	*	.	F	0.25	0.73
	Cys	146	A	.	.	.	.	T	.	-0.84	0.23	*	.	.	0.10	0.42
	Leu	147	A	A	.	.	.	.	.	-0.58	0.43	*	.	.	-0.60	0.17
	Gln	148	A	A	.	.	.	.	.	-0.27	0.53	*	.	.	-0.60	0.10
	Leu	149	A	A	.	.	.	.	.	-0.57	0.53	*	*	.	-0.30	0.32
	Ile	150	A	A	.	.	.	.	.	-0.57	0.34	*	.	.	0.30	0.52
	Ala	151	.	A	.	.	.	.	C	-0.21	-0.34	.	*	.	1.40	0.52
	Asp	152	.	.	.	.	T	T	.	0.39	-0.26	.	*	F	2.45	0.91
	Ser	153	.	.	.	.	.	T	C	0.08	-0.51	.	.	F	3.00	2.00
	Glu	154	.	.	.	.	.	T	C	-0.00	-0.71	.	.	F	2.70	2.86
	Thr	155	.	.	.	.	.	T	C	0.89	-0.53	*	.	F	2.40	1.20
	Pro	156	.	.	.	B	.	.	C	1.52	-0.13	*	.	F	1.56	1.55
	Thr	157	.	.	.	B	T	.	.	1.18	-0.51	*	.	F	1.92	1.79
	Ile	158	A	.	.	B	.	.	.	1.18	-0.09	.	.	F	1.08	1.23
	Gln	159	.	.	.	.	T	T	.	0.93	-0.19	.	.	F	2.04	1.07
	Lys	160	.	.	.	.	T	T	.	0.93	0.14	*	.	F	1.60	1.16
	Gly	161	.	.	.	.	T	T	.	0.44	0.14	*	.	F	1.44	2.38
	Ser	162	.	.	.	.	T	T	.	-0.10	0.24	*	.	F	1.28	1.19
	Tyr	163	.	.	.	B	T	.	.	0.58	0.49	*	.	.	0.12	0.44
	Thr	164	.	.	B	B	.	.	.	0.29	0.91	*	.	.	-0.44	0.69
	Phe	165	.	.	B	B	.	.	.	-0.57	1.40	*	.	.	-0.60	0.54
	Val	166	.	.	B	B	.	.	.	-1.03	1.70	.	.	.	-0.60	0.29
	Pro	167	.	.	B	B	.	.	.	-1.03	1.63	.	.	.	-0.60	0.16
	Trp	168	A	.	.	B	.	.	.	-1.49	1.53	.	*	.	-0.60	0.25
	Leu	169	A	.	.	B	.	.	.	-1.13	1.53	*	.	.	-0.60	0.29
	Leu	170	A	.	.	B	.	.	.	-0.32	0.89	*	.	.	-0.30	0.38
	Ser	171	A	.	.	.	.	.	.	0.19	0.46	*	.	.	0.20	0.71
	Phe	172	.	.	.	.	T	.	.	0.10	-0.03	*	.	.	1.80	0.85
	Lys	173	.	.	.	.	T	T	.	-0.20	-0.33	*	.	F	2.60	1.38
	Arg	174	.	.	.	.	.	T	C	-0.20	-0.51	.	.	F	3.00	1.04
	Gly	175	.	.	.	.	.	T	C	0.61	-0.21	.	.	F	2.25	0.99
	Ser	176	A	.	.	.	.	T	.	0.91	-1.00	*	.	F	2.05	0.86
	Ala	177	A	A	.	.	.	.	.	1.66	-1.00	*	.	F	1.35	0.76

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Leu	178	A	A	.	.	.	.	.	1.61	-1.00	.	.	F	1.20	1.54
Glu	179	A	A	.	.	.	.	.	1.50	-1.43	.	.	F	0.90	1.98
Glu	180	A	A	.	.	.	.	.	1.89	-1.41	*	.	F	0.90	3.16
Lys	181	A	A	.	.	.	.	.	1.30	-1.91	*	.	F	0.90	7.66
Glu	182	A	A	.	.	.	.	.	1.08	-1.91	.	.	F	0.90	3.10
Asn	183	A	A	.	.	.	.	.	1.03	-1.23	*	*	F	0.90	1.48
Lys	184	A	A	.	.	.	.	.	1.08	-0.59	*	.	F	0.75	0.55
Ile	185	A	A	.	.	.	.	.	1.08	-0.59	*	*	.	0.60	0.63
Leu	186	A	A	.	.	.	.	.	0.72	-0.59	*	*	.	0.60	0.68
Val	187	A	A	.	.	.	.	.	0.38	-0.50	.	*	.	0.30	0.49
Lys	188	A	A	.	.	.	.	.	0.13	-0.07	*	*	F	0.45	0.69
Glu	189	A	.	.	.	.	T	.	-0.61	0.00	*	*	F	0.40	1.32
Thr	190	.	.	.	.	T	T	.	-0.42	0.10	.	*	F	0.80	1.54
Gly	191	.	.	.	.	T	T	.	-0.50	0.24	*	.	F	0.65	0.67
Tyr	192	.	.	.	.	T	T	.	0.11	0.93	*	*	.	0.20	0.27
Phe	193	.	.	B	B	.	.	.	-0.28	1.69	.	.	.	-0.60	0.29
Phe	194	.	.	B	B	.	.	.	-0.28	1.63	.	*	.	-0.60	0.29
Ile	195	.	.	B	B	.	.	.	-0.82	1.60	.	.	.	-0.60	0.32
Tyr	196	.	.	B	B	.	.	.	-1.29	1.49	.	.	.	-0.60	0.28
Gly	197	.	.	.	B	T	.	.	-1.29	1.39	.	.	.	-0.20	0.26
Gln	198	.	.	.	B	T	.	.	-0.90	1.36	.	.	.	-0.20	0.59
Val	199	.	.	.	B	.	.	C	-0.20	1.16	.	.	.	-0.40	0.54
Leu	200	.	.	.	B	.	.	C	0.73	0.40	.	.	.	-0.10	0.92
Tyr	201	.	.	.	.	T	T	.	0.67	-0.03	.	.	.	1.25	1.06
Thr	202	.	.	.	.	T	T	.	0.77	0.06	.	.	F	0.80	2.06
Asp	203	.	.	.	.	T	T	.	0.18	0.17	.	.	F	0.80	3.91
Lys	204	A	.	.	.	.	T	.	0.43	-0.01	.	.	F	1.00	2.52
Thr	205	A	A	.	.	.	.	.	0.90	-0.16	.	.	F	0.60	1.73
Tyr	206	A	A	.	.	.	.	.	1.11	-0.21	.	.	.	0.45	1.03
Ala	207	A	A	.	.	.	.	.	0.61	0.29	.	.	.	-0.30	0.70
Met	208	A	A	.	.	.	.	.	-0.28	0.97	.	.	.	-0.60	0.40
Gly	209	A	A	.	B	.	.	.	-0.32	1.17	*	.	.	-0.60	0.18
His	210	A	A	.	B	.	.	.	0.10	0.81	*	.	.	-0.60	0.31
Leu	211	A	A	.	B	.	.	.	0.39	0.31	.	.	.	-0.30	0.61
Ile	212	A	A	.	B	.	.	.	1.02	-0.30	.	.	.	0.45	1.22
Gln	213	A	A	.	B	.	.	.	0.77	-0.73	.	*	.	0.75	1.80
Arg	214	A	A	.	B	.	.	.	1.08	-0.59	.	*	F	0.90	1.62
Lys	215	A	A	.	B	.	.	.	0.26	-0.77	*	*	F	0.90	3.14
Lys	216	A	A	.	B	.	.	.	0.37	-0.81	.	*	F	0.90	1.35
Val	217	.	A	B	B	.	.	.	0.91	-0.43	*	*	.	0.30	0.60
His	218	.	A	B	B	.	.	.	0.91	-0.00	.	*	.	0.30	0.29
Val	219	.	A	B	B	.	.	.	0.80	-0.00	*	*	.	0.30	0.25
Phe	220	.	.	B	B	.	.	.	-0.06	-0.00	*	.	.	0.30	0.57
Gly	221	A	.	.	B	.	.	.	-0.40	0.04	.	*	.	-0.30	0.35
Asp	222	A	.	.	.	.	.	.	-0.36	-0.07	*	.	.	0.50	0.63
Glu	223	A	.	.	.	.	.	.	-1.18	-0.03	*	.	.	0.50	0.60
Leu	224	A	.	.	B	.	.	.	-0.63	-0.17	.	.	.	0.30	0.45
Ser	225	A	.	.	B	.	.	.	-0.74	-0.11	.	.	.	0.30	0.39
Leu	226	A	.	.	B	.	.	.	-1.10	0.57	.	*	.	-0.60	0.18
Val	227	A	.	.	B	.	.	.	-0.99	1.36	.	*	.	-0.60	0.19
Thr	228	A	.	.	B	.	.	.	-1.66	0.67	*	*	.	-0.60	0.28
Leu	229	A	.	.	B	.	.	.	-1.73	0.86	*	.	.	-0.60	0.18
Phe	230	A	.	.	B	.	.	.	-1.43	0.86	*	.	.	-0.60	0.17
Arg	231	A	.	.	B	.	.	.	-0.62	0.61	*	.	.	-0.60	0.21
Cys	232	.	.	.	B	T	.	.	-0.37	0.53	*	.	.	-0.20	0.41
Ile	233	.	.	.	B	T	.	.	-0.27	0.46	*	.	.	-0.20	0.46
Gln	234	.	.	.	B	T	.	.	0.54	0.10	*	.	.	0.10	0.37
Asn	235	.	.	.	B	.	.	C	0.93	0.10	*	.	.	0.05	1.19
Met	236	.	.	.	B	.	.	C	0.01	0.01	*	.	F	0.20	2.44

Table I (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Pro	237	.	.	.	B	.	.	C	0.47	0.01	*	.	F	0.44	1.16
Glu	238	.	.	.	.	T	.	.	1.36	0.04	*	.	F	1.08	1.12
Thr	239	.	.	.	.	.	.	C	1.36	0.04	*	.	F	1.12	1.82
Leu	240	.	.	.	.	.	.	C	1.06	-0.17	*	.	F	1.96	1.89
Pro	241	.	.	.	.	T	.	.	0.99	-0.21	.	.	F	2.40	1.46
Asn	242	.	.	.	.	T	.	.	0.96	0.36	.	.	F	1.41	0.54
Asn	243	.	.	.	.	T	T	.	0.66	0.63	.	.	F	1.22	1.03
Ser	244	.	.	.	.	T	T	.	0.38	0.33	.	.	F	1.13	0.89
Cys	245	.	.	.	.	T	T	.	0.84	0.40	.	.	.	0.74	0.56
Tyr	246	.	.	.	.	T	T	.	0.17	0.43	.	.	.	0.20	0.35
Ser	247	A	.	.	.	.	.	.	-0.42	0.71	.	.	.	-0.40	0.18
Ala	248	A	A	.	.	.	.	.	-0.38	0.83	.	.	.	-0.60	0.34
Gly	249	A	A	.	.	.	.	.	-0.89	0.26	.	.	.	-0.30	0.43
Ile	250	A	A	.	.	.	.	.	-0.22	0.19	*	.	.	-0.30	0.27
Ala	251	A	A	.	.	.	.	.	0.02	-0.20	*	.	.	0.30	0.46
Lys	252	A	A	.	.	.	.	.	-0.02	-0.70	.	.	.	0.60	0.80
Leu	253	A	A	.	.	.	.	.	0.57	-0.70	.	.	F	0.90	1.13
Glu	254	A	A	.	.	.	.	.	0.91	-1.39	.	.	F	0.90	1.87
Glu	255	A	A	.	.	.	.	.	0.99	-1.89	.	.	F	0.90	1.62
Gly	256	A	A	.	.	.	.	.	1.58	-1.20	.	*	F	0.90	1.62
Asp	257	A	A	.	.	.	.	.	0.72	-1.49	.	*	F	0.90	1.62
Glu	258	A	A	.	.	.	.	.	0.94	-0.80	*	*	F	0.75	0.77
Leu	259	A	A	.	.	.	.	.	0.06	-0.30	*	*	.	0.30	0.79
Gln	260	A	A	.	.	.	.	.	-0.16	-0.04	*	.	.	0.30	0.33
Leu	261	A	A	.	.	.	.	.	0.30	0.39	*	.	.	-0.30	0.30
Ala	262	A	A	.	.	.	.	.	0.30	0.39	*			-0.30	0.70
Ile	263	A	A	.	.	.	.	.	0.30	-0.30	.	*	.	0.30	0.70
Pro	264	A	.	.	.	.	T	.	0.52	-0.30	.	*	F	1.00	1.37
Arg	265	A	.	.	.	.	T	.	0.52	-0.49	.	*	F	1.00	1.37
Glu	266	A	.	.	.	.	T	.	0.44	-0.59	*	*	F	1.30	3.38
Asn	267	A	.	.	.	.	T	.	0.73	-0.59	*	*	F	1.30	1.53
Ala	268	A	.	.	.	.	.	.	0.81	-0.63	*	*	.	0.95	1.05
Gln	269	A	.	.	.	.	.	.	1.02	0.06	*	*		-0.10	0.50
Ile	270	A	.	.	.	.	.	.	0.57	0.06	.	*	.	0.15	0.52
Ser	271	.	.	.	.	.	.	C	0.57	0.09	.	*	.	0.60	0.51
Leu	272	.	.	.	.	.	.	C	-0.29	-0.41	.	*	F	1.60	0.49
Asp	273	.	.	.	.	T	T	.	-0.01	-0.17	.	*	F	2.25	0.52
Gly	274	.	.	.	.	T	T	.	-0.71	-0.37	.	*	F	2.50	0.56
Asp	275	.	.	.	.	T	T	.	-0.52	0.03	.	*	F	1.65	0.59
Val	276	A	.	.	.	.	T	.	-0.57	0.13	.	*	F	1.00	0.30
Thr	277	A	.	.	B	.	.	.	-0.34	0.56	.	*	.	-0.10	0.30
Phe	278	A	.	.	B	.	.	.	-1.16	0.63	.	*	.	-0.35	0.18
Phe	279	A	.	.	B	.	.	.	-0.77	1.31	.	*	.	-0.60	0.20
Gly	280	A	A	.	.	.	.	.	-1.58	0.67	.	*	.	-0.60	0.28
Ala	281	A	A	.	.	.	.	.	-1.53	0.87	.	*	.	-0.60	0.27
Leu	282	A	A	.	.	.	.	.	-1.61	0.77	*	.	.	-0.60	0.26
Lys	283	A	A	.	.	.	.	.	-1.30	0.41	*	.	.	-0.60	0.33
Leu	284	A	A	.	.	.	.	.	-0.99	0.41	.	.	.	-0.60	0.42
Leu	285	A	A	.	.	.	.	.	-1.03	0.34	*	.	.	-0.30	0.65

Additional preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding one or more epitope-bearing portions of Neutrokine-α. In particular, such nucleic acid fragments of the present invention included nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about Phe-115 to about Leu-147, from about Ile-150 to about Tyr-163, from about Ser-171 to about Phe-194, from about Glu 223 to about Tyr-247, and from about Ser-271 to about Phe-278, of the amino acid sequence of SEQ ID NO:2. Polypeptide fragments which bear antigenic epitopes of the Neutrokine-α may be easily determined by one of skill in the art using the above-described analysis of the Jameson-Wolf antigenic index, as shown in Figure 3. Methods for determining other such epitope-bearing portions of Neutrokine α are described in detail below.

Additional preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding one or more epitope-bearing portions of Neutrokine αSV. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about Pro-32 to about Leu-47, from about Glu-116 to about Ser-143, from about Phe-153 to about Tyr-173, from about Pro-218 to about Tyr-227, from about Ser-252 to about Thr-258, from about Ala-232 to about Gln-241; from about Ile-244 to about Ala-249; and from about Ser-252 to about Val-257, of the amino acid sequence of SEQ ID NO:19. Polypeptide fragments which bear antigenic epitopes of the Neutrokine-α may be easily determined by one of skill in the art using the above-described analysis of the Jameson-Wolf antigenic index. Methods for determining other such epitope-bearing portions of Neutrokine-αSV are described in detail below.

In specific embodiments, the polynucleotides of the invention are less than 100000 kb, 50000 kb, 10000 kb, 1000 kb, 500 kb, 400 kb, 350 kb, 300 kb, 250 kb, 200 kb, 175 kb, 150 kb, 125 kb, 100 kb, 75 kb, 50 kb, 40 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, 7.5 kb, or 5 kb in length.

In further embodiments, polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of Neutrokine-α coding sequence, but consist of less than or equal to 1000 kb, 500 kb, 250 kb, 200 kb, 150 kb, 100 kb, 75 kb, 50 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, or 5 kb of genomic DNA that flanks the 5' or 3' coding nucleotide set forth in Figures 1A and 1B (SEQ ID NO:1) or Figures 5A and 5B (SEQ ID NO:18). In further embodiments,

polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of Neutrokine-α coding sequence, but do not comprise all or a portion of any Neutrokine-α intron. In another embodiment, the nucleic acid comprising Neutrokine-α coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the Neutrokine-α gene in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

In another embodiment, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the sequence complementary to the coding and/or noncoding sequence depicted in Figures 1A and 1B (SEQ ID NO:1), the sequence of the cDNA clone contained in the deposit having ATCC accession no. 97768, the sequence complementary to the coding sequence and/or noncoding sequence depicted in Figures 5A and 5B (SEQ ID NO:18), the sequence of the cDNA clone contained in the deposit having ATCC accession no. 203518, or fragments of these sequences, as described herein. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 40, 50, or 60) nucleotides, and even more preferably about any integer in the range of 30-70 or 80-150 nucleotides, or the entire length of the reference polynucleotide. These have uses, which include, but are not limited to, diagnostic probes and primers as discussed above and in more detail below. By a portion of a polynucleotide of "at least about 20 nt in length," for example, is intended to include the particularly recited ranges, larger or smaller by several (i.e. 5, 4, 3, 2, 1, or 0) amino acids, at either extreme or at both extremes of the nucleotide sequence of the reference polynucleotide (e.g., the sequence of one

or both of the deposited cDNAs, the complementary strand of the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1), or the complementary strand of the nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the Neutrokine-a cDNA shown in Figures 1A and 1B (SEQ ID NO:1) or the 3' terminal poly(A) tract of the Neutrokine-aSV cDNA shown in Figures 5A and 5B (SEQ ID NO:18)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

As indicated, nucleic acid molecules of the present invention which encode a Neutrokine-a polypeptide or a Neutrokine-aSV polypeptide may include, but are not limited to, polynucleotides encoding the amino acid sequence of the respective extracellular domains of the polypeptides, by themselves; and the coding sequence for the extracellular domains of the respective polypeptides and additional sequences, such as those encoding the intracellular and transmembrane domain sequences, or a pre-, or pro- or prepro-protein sequence; the coding sequence of the respective extracellular domains of the polypeptides, with or without the aforementioned additional coding sequences.

Also encoded by nucleic acids of the invention are the above protein sequences together with additional, non-coding sequences, including for example, but not limited to, introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example, ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this embodiment of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824

(1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson et al., *Cell* 37: 767 (1984). As discussed below, other such fusion proteins include the Neutrokine-a or the Neutrokine-aSV polypeptides fused to Fc at the N- or C-terminus.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the Neutrokine-a or Neutrokine-aSV polypeptides of SEQ ID NO:2. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the Neutrokine-a and/or Neutrokine-aSV polypeptides or portions thereof. Also especially preferred in this regard are conservative substitutions.

Additional embodiments of the invention are directed to isolated nucleic acid molecules comprising a polynucleotide which encodes the amino acid sequence of a Neutrokine-a and/or Neutrokine-aSV polypeptide (e.g., a Neutrokine-a and/or Neutrokine-aSV polypeptide fragment described herein) having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions, 10-20 conservative amino acid substitutions, 5-10 conservative amino acid substitutions, 1-5 conservative amino acid substitutions, 3-5 conservative amino acid substitutions, or 1-3 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Neutrokine-a and/or Neutrokine-aSV polypeptide to have an amino acid

sequence which contains not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

Most highly preferred are nucleic acid molecules encoding the extracellular domain of the protein having the amino acid sequence shown in Figures 1A and 1B (SEQ ID NO:2) or the extracellular domain of the Neutrokine-a amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 97768. Further embodiments include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding the Neutrokine-a polypeptide having the complete amino acid sequence in Figures 1A and 1B (i.e., positions 1 to 285 of SEQ ID NO:2); (b) a nucleotide sequence encoding the Neutrokine-a polypeptide having the complete amino acid sequence in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions 2 to 285 of SEQ ID NO:2); (c) a fragment of the polypeptide of (b) having Neutrokine-a functional activity (e.g., antigenic or biological activity); (d) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokine-a polypeptide having the amino acid sequence at positions 73-285 in Figures 1A and 1B (SEQ ID NO:2); (e) a nucleotide sequence encoding the Neutrokine-a polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (f) a nucleotide sequence encoding the extracellular domain of the Neutrokine-a polypeptide having the amino acid sequence encoded by the cDNA contained in the deposit having ATCC accession number 97768; and (g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), or (g) above.

A further embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of a Neutrokine-a polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably not more than 30 conservative amino acid substitutions, and still even more preferably not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Neutrokine-a polypeptide to have an amino acid sequence which contains not more than 7-10, 5-10, 3-7, 3-5, 2-5, 1-5, 1-3, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

Also most highly preferred are nucleic acid molecules encoding the extracellular domain of the protein having the amino acid sequence shown in Figures 5A and 5B (SEQ ID NO:19) or the extracellular domain of the Neutrokine-aSV amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 203518. Further embodiments include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding the Neutrokine-aSV polypeptide having the complete amino acid sequence in Figures 5A and 5B (i.e., positions 1 to 266 of SEQ ID NO:19); (b) a nucleotide sequence encoding the Neutrokine-aSV polypeptide having the complete amino acid sequence in SEQ ID NO:19 excepting the N-terminal methionine (i.e., positions 2 to 266 of SEQ ID NO:2); (c) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokine-aSV polypeptide having the amino acid sequence at positions 73-285 in Figures 5A and 5B (SEQ ID NO:19); (d) a nucleotide sequence encoding the Neutrokine-aSV polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 203518; (e) a nucleotide sequence encoding the extracellular domain of the Neutrokine-aSV polypeptide having the amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 203518; and (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e), above.

Further, the invention includes a polynucleotide comprising a sequence at least 95% identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence from nucleotide 1 to nucleotide 1082 in Figures 1A and 1B (SEQ ID NO:1), preferably excluding the nucleotide sequences determined from the abovelisted cDNA clones and the nucleotide sequences from nucleotide 797 to 1082, 810 to 1082, and 346 to 542. In this context "about" includes the particularly recited ranges, larger or smaller by several (i.e. 5, 4, 3, 2 or 1) amino acids, at either extreme or at both extremes.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a Neutrokine-a and/or Neutrokine-aSV polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five mismatches per each 100

nucleotides of the reference nucleotide sequence encoding the Neutrokine-a and/or Neutrokine-aSV polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The reference (query) sequence may be the entire nucleotide sequence encoding Neutrokine-α or Neutrokine-αSV, as shown in Figures 1A and 1B (SEQ ID NO:1) and Figures 5A and 5B (SEQ ID NO:18), respectively, or any Neutrokine-α or Neutrokine-αSV polynucleotide fragment as described herein.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequences shown in Figures 1A and 1B, or the nucleotide sequences shown in Figures 5A and 5B, or to the nucleotides sequence of the deposited cDNA clones, or fragments thereof, can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman to find the best segment of homology between two sequences (*Advances in Applied Mathematics* 2:482-489 (1981)). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by

converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. A determination of whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of this embodiment. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences (i.e., polynucleotides) disclosed herein (e.g., those disclosed in Figures 1A and 1B (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs), irrespective of whether they encode a polypeptide having Neutrokine-α and/or Neutrokine-αSV functional activity (e.g., biological activity). In addition, the present application is also directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 5A and 5B (SEQ ID NO:18) or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having Neutrokine-αSV activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having Neutrokine-α and/or Neutrokine-αSV activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having Neutrokine-α and/or Neutrokine-αSV activity include, inter alia, (1) isolating the Neutrokine-α and/or Neutrokine-αSV gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the Neutrokine-α and/or Neutrokine-αSV gene, as described in Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and Northern Blot analysis for detecting Neutrokine-α and/or Neutrokine-αSV mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences disclosed herein (e.g., the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1) and the nucleic acid sequence of the deposited cDNAs, or fragments thereof), which do, in fact, encode a polypeptide having Neutrokine-α and/or Neutrokine-αSV polypeptide functional activity (e.g., biological activity). Also preferred are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 5A and 5B (SEQ ID NO:18) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having Neutrokine-α and/or Neutrokine-αSV polypeptide functional activity (e.g., biological activity).

By "a polypeptide having Neutrokine-α polypeptide functional activity" (e.g., biological activity) and "a polypeptide having Neutrokine-αSV

polypeptide functional activity" (e.g., biological activity) are intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the extracellular domain or the full-length Neutrokine-a or Neutrokine-aSV polypeptides of the invention, as measured in a particular functional assay (e.g., immunological or biological assay). For example, Neutrokine-a and/or Neutrokine-aSV polypeptide functional activity can be measured by the ability of a polypeptide sequence described herein to form multimers (e.g., homodimers and homotrimers) with the complete Neutrokine-a and/or Neutrokine- aSV or extracellular domain of Neutrokine-a and/or Neutrokine- aSV, and to bind a Neutrokine-a and/or Neutrokine-aSV ligand (e.g., DR5 (*See*, International Publication No. WO 98/41629), TR10 (*See*, International Publication No. WO 98/54202), 312C2 (*See*, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (*See*, U.S. Application Serial No. 09/176,200)). Neutrokine-a and/or Neutrokine-aSV polypeptide functional activity can be also be measured by determining the ability of a polypeptide of the invention to induce lymphocyte (e.g., B cell) proliferation, differentiation or activation. These functional assays can be routinely performed using techniques described herein (e.g., see Example 6) and otherwise known in the art. Additionally, Neutrokine-a or Neutrokine-aSV polypeptides of the present invention modulate cell proliferation, cytotoxicity and cell death. An *in vitro* cell proliferation, cytotoxicity and cell death assay for measuring the effect of a protein on certain cells can be performed by using reagents well known and commonly available in the art for detecting cell replication and/or death. For instance, numerous such assays for TNF-related protein activities are described in the various references in this disclosure. Briefly, an example of such an assay involves collecting human or animal (e.g., mouse) cells and mixing with (1) transfected host cell-supernatant containing Neutrokine-a protein (or a candidate polypeptide) or (2) nontransfected host cell-supernatant control, and measuring the effect on cell numbers or viability after incubation of certain period of time. Such cell proliferation modulation activities as can be measure in this type of assay are useful for treating tumor, tumor metastasis, infections, autoimmune diseases inflammation and other immune-related diseases.

Neutrokine-a and Neutrokine-aSV modulate cell proliferation and differentiation in a dose-dependent manner in the above-described assay. Accordingly, it is preferred that "a polypeptide having Neutrokine-a polypeptide functional activity" (e.g., biological activity) and "a polypeptide having Neutrokine-aSV polypeptide functional activity" (e.g., biological activity)

includes polypeptides that also exhibit any of the same cell modulatory (particularly immunomodulatory) activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the Neutrokine-a and/or Neutrokine-aSV polypeptides, preferably, "a polypeptide having Neutrokine-a polypeptide functional activity" and "a polypeptide having Neutrokine-aSV polypeptide functional activity" will exhibit substantially similar dose-dependence in a given activity as compared to the Neutrokine-a and/or Neutrokine-aSV polypeptides (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference Neutrokine-a and/or Neutrokine-aSV polypeptides).

In certain preferred embodiments, "a polypeptide having Neutrokine-a polypeptide functional activity" (e.g., biological activity) and "a polypeptide having Neutrokine-aSV polypeptide functional activity" (e.g., biological activity) includes polypeptides that also exhibit any of the same B cell (or other cell type) modulatory (particularly immunomodulatory) activities described in Figures 8A, 8B, 9A, 9B, 10, 11, 12A, and 12B, and in Example 6.

Like other members of TNF family, Neutrokine-a exhibits activity on leukocytes including, for example, monocytes, lymphocytes (e.g., B cells) and neutrophils. For this reason Neutrokine-a is active in directing the proliferation, differentiation and migration of these cell types. Such activity is useful for immune enhancement or suppression, myeloprotection, stem cell mobilization, acute and chronic inflammatory control and treatment of leukemia. Assays for measuring such activity are known in the art. For example, see Peters et al., Immun. Today 17:273 (1996); Young et al., J. Exp. Med. 182:1111 (1995); Caux et al., Nature 390:258 (1992); and Santiago-Schwarz et al., Adv. Exp. Med. Biol. 378:7 (1995)."

Moreover, Neutrokine-aSV also exhibits activity on leukocytes including for example monocytes, lymphocytes and neutrophils. For this reason Neutrokine-aSV is active in directing the proliferation, differentiation and migration of these cell types. Such activity is useful for immune enhancement or suppression, myeloprotection, stem cell mobilization, acute and chronic inflammatory control and treatment of leukemia. Assays for measuring such activity are known in the art. For example, see Peters *et al., Immun. Today 17*:273 (1996); Young *et al., J. Exp. Med. 182*:1111 (1995); Caux *et al., Nature 390*:258 (1992); and Santiago-Schwarz *et al., Adv. Exp. Med. Biol. 378*:7 (1995)."

Of course, due to the degeneracy of the genetic code, one of ordinary

skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence contained in cDNA clone deposited in ATCC accession no. 97768, or the nucleic acid sequence shown in Figures 1A and 1B (SEQ ID NO:1), or fragments thereof, will encode a polypeptide "having Neutrokine-a polypeptide functional activity" (e.g., biological activity). One of ordinary skill in the art will also immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence contained in cDNA clone deposited in ATCC accession no. 203518 or the nucleic acid sequence shown in Figures 5A and 5B (SEQ ID NO:18) will encode a polypeptide "having Neutrokine-aSV polypeptide functional activity" (e.g., biological activity). In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having Neutrokine-a and/or Neutrokine- aSV activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

A further embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of a Neutrokine-aSV polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably not more than 30 conservative amino acid substitutions, and still even more preferably not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Neutrokine-aSV polypeptide to have an amino acid sequence which contains not more than 7-10, 5-10, 3-7, 3-5, 2-5, 1-5, 1-3, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

Polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:2, encoded by the cDNA contained in the deposited clone, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited clone, or

shown in Figures 1A and 1B (SEQ ID NO:1) or the complementary strand thereto. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 50, 51 to 100, 101 to 150, 151 to 200, 201 to 250, and/or 251 to 285 of SEQ ID NO:2. Moreover, polypeptide fragments can be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175 or 200 amino acids in length.

In specific embodiments, polypeptide fragments of the invention comprise, or alternatively consist of, amino acid residues: 1-46, 31-44, 47-72, 73-285, 73-83, 94-102, 148-152, 166-181, 185-209, 210-221, 226-237, 244-249, 253-265, and/or 277-284, as depicted in Figures 1A and 1B (SEQ ID NO:2). Polynucleotides encoding these polypeptides are also encompassed by the invention.

It will be recognized by one of ordinary skill in the art that mutations targeted to regions of a Neutrokine-α polypeptide of the invention which encompass the nineteen amino acid residue insertion which is not found in the Neutrokine-αSV polypeptide sequence (i.e., amino acid residues Val-142 through Lys-160 of the sequence presented in Figures 1A and 1B and in SEQ ID NO:2) may affect the observed biological activities of the Neutrokine-α polypeptide. More specifically, a partial, non-limiting and non-exclusive list of such residues of the Neutrokine-α polypeptide sequence which may be targeted for mutation includes the following amino acid residues of the Neutrokine-α polypeptide sequence as shown in SEQ ID NO:2: V-142; T-143; Q-144; D-145; C-146; L-147; Q-148; L-149; I-150; A-151; D-152; S-153; E-154; T-155; P-156; T-157; I-158; Q-159; and K-160. Polynucleotides encoding Neutrokine-α polypeptides which have one or more mutations in the region from V-142 through K-160 of SEQ ID NO:2 are contemplated.

Similarly, polynucleotides encoding polypeptides which contain all or some portion of the region V-142 through K-160 of SEQ ID NO:2 are likely to be valuable diagnostic and therapeutic polynucleotides with regard to detecting and/or altering expression of either Neutrokine-α or Neutrokine-αSV polynucleotides. In addition, polynucleotides which span the junction of amino acid residues T-141 and G-142 of the Neutrokine-αSV polypeptide shown in SEQ ID NO:19 (in between which the V-142 through K-160 amino acid sequence of Neutrokine-α is apparently inserted), are also likely to be useful both diagnostically and therapeutically. Such T-141/G-142 spanning

polynucleotides will exhibit a much higher likelihood of hybridization with Neutrokine-aSV polynucleotides than with Neutrokine-a polynucleotides. A partial, non-limiting, non-exclusive list of such Neutrokine-aSV polypeptides which are encoded by polynucleotides of the invention includes the following: G-121 through E-163; E-122 through E-163; G-123 through E-163; N-124 through E-163; S-125 through E-163; S-126 through E-163; Q-127 through E-163; N-128 through E-163; S-129 through E-163; R-130 through E-163; N-131 through E-163; K-132 through E-163; R-133 through E-163; A-134 through E-163; V-135 through E-163; Q-136 through E-163; G-137 through E-163; P-138 through E-163; E-139 through E-163; E-140 through E-163; T-141 through E-163; G-142 through E-163; S-143 through E-163; Y-144 through E-163; T-145 through E-163; F-146 through E-163; V-147 through E-163; P-148 through E-163; W-149 through E-163; L-150 through E-163; L-151 through E-163; S-152 through E-163; F-153 through E-163; K-154 through E-163; R-155 through E-163; G-156 through E-163; S-157 through E-163; A-158 through E-163; L-159 through E-163; E-160 through E-163; E-161 through E-163; K-162 through E-163; G-121 through K-162; G-121 through E-161; G-121 through E-160; G-121 through L-159; G-121 through A-158; G-121 through S-157; G-121 through G-156; G-121 through R-155; G-121 through K-154; G-121 through F-153; G-121 through S-152; G-121 through L-151; G-121 through L-150; G-121 through W-149; G-121 through P-148; G-121 through V-147; G-121 through F-146; G-121 through T-145; G-121 through Y-144; G-121 through S-143; G-121 through G-142; G-121 through T-141; G-121 through E-140; G-121 through E-139; G-121 through P-138; G-121 through G-137; G-121 through Q-136; G-121 through V-135; G-121 through A-134; G-121 through R-133; G-121 through K-132; G-121 through N-131; G-121 through R-130; G-121 through S-129; G-121 through N-128; G-121 through Q-127; G-121 through S-126; G-121 through S-125; G-121 through N-124; G-121 through G-123; and G-121 through E-122 of SEQ ID NO:19.

Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, or which are otherwise engineered to produce the polypeptides of the invention, and the production of Neutrokine-a and/or Neutrokine-aSV polypeptides, or fragments thereof, by recombinant

techniques.

In one embodiment, the polynucleotides of the invention are joined to a vector (e.g., a cloning or expression vector). The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells. The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Introduction of the vector construct into the host cell can be effected by techniques known in the art which include, but are not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986).

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, for example, stabilization or simplified purification of expressed recombinant product.

In one embodiment, the DNA of the invention is operatively associated with an appropriate heterologous regulatory element (e.g., promoter or enhancer), such as, the phage lambda PL promoter, the *E. coli lac, trp, phoA*, and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells;

fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed. Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice. As a representative, but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Among vectors preferred for use in bacteria include pHE4-5 (ATCC Accession No. 209311; and variations thereof), pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., *supra*; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a,

pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman (*Cell* 23:175 (1981)), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., Neutrokine-α coding sequence), and/or to include genetic material (e.g.,

heterologous polynucleotide sequences) that is operably associated with Neutrokine-α polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous Neutrokine-α polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous Neutrokine-α polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

The host cells described *infra* can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, cell-free translation systems can also be employed to produce the polypeptides of the invention using RNAs derived from the DNA constructs of the present invention.

The polypeptide of the invention may be expressed or synthesized in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals, but also additional heterologous functional regions. Such a fusion protein can be made by ligating polynucleotides of the invention and the desired nucleic acid sequence encoding the desired amino acid sequence to each other, by methods known in the art, in the proper reading frame, and expressing the fusion protein product by methods known in the art. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins

comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5 has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995) and K. Johanson *et al., J. Biol. Chem. 270*:9459-9471 (1995).

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller, M., et al., 1984, Nature 310:105-111). For example, a peptide corresponding to a fragment of the complete Neutrokine-α or Neutrokine-αSV polypeptides of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the Neutrokine-α or Neutrokine-αSV polynucleotide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer

amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses Neutrokine-α or Neutrokine-αSV polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of Neutrokine-α or Neutrokine-αSV which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity,

the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The Neutrokine-α and/or Neutrokine-αSV polypeptides can be recovered and purified by known methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite

chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Neutrokine-α Polypeptides

The Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention, their preparation, and compositions (preferably, pharmaceutical compositions) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention (including Neutrokine-α and/or Neutrokine-αSV fragments, variants, and fusion proteins, as described herein). These homomers may contain Neutrokine-α and/or Neutrokine-αSV polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only Neutrokine-α and/or Neutrokine-αSV polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing Neutrokine-α and/or Neutrokine-αSV polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing Neutrokine-α and/or Neutrokine-αSV polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing Neutrokine-α and/or Neutrokine-αSV polypeptides having identical or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing heterologous polypeptides (i.e., polypeptides of a different protein) in addition to the Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention. In a

specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:2 or SEQ ID NO:19, or contained in the polypeptide encoded by the clones deposited in connection with this application). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a Neutrokine-α and/or Neutrokine-αSV fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Neutrokine-α-Fc and/or Neutrokine-αSV -Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety).

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

In one embodiment, the invention provides an isolated Neutrokine-α polypeptide having the amino acid sequence encoded by the cDNA clone

contained in ATCC No. 97768, or the amino acid sequence in Figures 1A and 1B (SEQ ID NO:2), or a peptide or polypeptide comprising a portion (i.e., a fragment) of the above polypeptides. In another embodiment, the invention provides an isolated Neutrokine-αSV polypeptide having the amino acid encoded by the cDNA clone contained in ATCC No. 203518, or the amino acid sequence in Figures 5A and 5B (SEQ ID NO:19), or a peptide or polypeptide comprising a portion (i.e, fragment) of the above polypeptides.

Polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:2, encoded by the cDNA contained in the plasmid having ATCC accession number 97768, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited clone, or the complementary strand of the nucleotide sequence shown in Figures 1A-B (SEQ ID NO:1.

Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:19, encoded by the cDNA contained in the plasmid having ATCC accession number 203518, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited clone, or the complementary strand of the nucleotide sequence shown in Figures 5A-B (SEQ ID NO:18).

Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 15, 16-30, 31-46, 47-55, 56-72, 73-104, 105-163, 163-188, 186-210 and 210-284 of the amino acid sequence disclosed in SEQ ID NO:2. Additional representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 143, 1-150, 47-143, 47-150, 73-143, 73-150, 100-150, 140-145, 142-148, 140-150, 140-200, 140-225, and 140-266 of the amino acid sequence disclosed in SEQ ID NO:19. Moreover, polypeptide fragments can be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175 or 200 amino acids in length. In this context, "about" means several, a few, 5, 4, 3, 2 or 1. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Additional preferred embodiments encompass polypeptide fragments comprising, or alternatively consisting of, the predicted intracellular domain of

Neutrokine-a (amino acid residues 1-46 of SEQ ID NO:2), the predicted transmembrane domain of Neutrokine-a (amino acid residues 47-72 of SEQ ID NO:2), the predicted extracellular domain of Neutrokine-a (amino acid residues 73-285 of SEQ ID NO:2), the predicted TNF conserved domain of Neutrokine-α (amino acids 191 to 284 of SEQ ID NO:2), and a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain fused to the predicted extracellular domain of Neutrokine-a (amino acid residues 1-46 fused to amino acid residues 73-285 of SEQ ID NO:2).

Further additional preferred embodiments encompass polypeptide fragments comprising, or alternatively consisting of, the predicted intracellular domain of Neutrokine-αSV (amino acid residues 1-46 of SEQ ID NO:19), the predicted transmembrane domain of Neutrokine-aSV (amino acid residues 47-72 of SEQ ID NO:19), the predicted extracellular domain of Neutrokine-aSV (amino acid residues 73-266 of SEQ ID NO:19), the predicted TNF conserved domain of Neutrokine-αSV (amino acids 172 to 265 of SEQ ID NO:19), and a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain fused to the predicted extracellular domain of Neutrokine-αSV (amino acid residues 1-46 fused to amino acid residues 73-266 of SEQ ID NO:19).

Additional embodiments encompass Neutrokine-α and/or Neutrokine-αSV polypeptide fragments comprising functional regions of polypeptides of the invention, such as the Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and coil-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index set out in Figures 3 and 6 and in Table I and as described herein. In a preferred embodiment, the polypeptide fragments of the invention are antigenic. The data presented in columns VIII, IX, XIII, and XIV of Table I can be used to routinely determine regions of Neutrokine-a which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response. Among highly preferred fragments of the invention are those that comprise regions of Neutrokine-a and/or Neutrokine-aSV that

combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In another embodiment, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. Polynucleotides encoding these peptides or polypeptides are also encompassed by the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R. A. (1983) "Antibodies that react with predetermined sites on proteins", *Science, 219*:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate Neutrokine-α- and/or Neutrokine-αSV-specific antibodies include: a polypeptide comprising amino acid residues from about Phe-115 to about Leu-147 in Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ile-150 to about Tyr-163 in Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ser-171 to

about Phe-194 in Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Glu-223 to about Tyr-247 in Figures 1A and 1B (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about Ser-271 to about Phe-278 in Figures 1A and 1B (SEQ ID NO:2). These polypeptide fragments have been determined to bear antigenic epitopes of the Neutrokine-α polypeptide by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 3 and Table I, above.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate Neutrokine-α- and/or Neutrokine-αSV-specific antibodies include: a polypeptide comprising amino acid residues from about Pro-32 to about Leu-47 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising amino acid residues from about Glu-116 to about Ser-143 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising amino acid residues from about Phe-153 to about Tyr-173 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising amino acid residues from about Pro-218 to about Tyr-227 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising amino acid residues from about Ala-232 to about Gln-241 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising amino acid residues from about Ile-244 to about Ala-249 in Figures 5A and 5B (SEQ ID NO:19); and a polypeptide comprising amino acid residues from about Ser-252 to about Val-257 in Figures 5A and 5B (SEQ ID NO:19). Polynucleotides encoding these polypeptides are also encompassed by the invention.

These polypeptide fragments have been determined to bear antigenic epitopes of the Neutrokine-αSV polypeptide by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 6 and a tabular representation of the data presented in Figure 6 generated by the Protean component of the DNA*STAR computer program (as set forth above).

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. See, e.g., Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA* 82:5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U. S. Patent No. 4,631,211 to Houghten et al. (1986).

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985). Immunogenic epitope-bearing peptides of the invention,

i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen et al., *supra*. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

As one of skill in the art will appreciate, Neutrokine-α and/or Neutrokine-αSV polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker *et al., Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric Neutrokine-α and/or Neutrokine-αSV polypeptides or polypeptide fragments alone (Fountoulakis *et al., J. Biochem.* 270:3958-3964 (1995)).

To improve or alter the characteristics of Neutrokine-α and/or Neutrokine-αSV polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than

the corresponding natural polypeptide, at least under certain purification and storage conditions. For instance, for many proteins, including the extracellular domain or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., J. Biol. Chem., 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing.

In the present case, since the protein of the invention is a member of the TNF polypeptide family, deletions of N-terminal amino acids up to the Gly (G) residue at position 191 in Figures 1A and 1B (SEQ ID NO:2) may retain some biological activity such as, for example, the ability to stimulate lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and cytotoxicity to appropriate target cells. Polypeptides having further N-terminal deletions including the Gly (G) residue would not be expected to retain biological activities because it is known that this residue in TNF-related polypeptides is in the beginning of the conserved domain required for biological activities. However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or extracellular domain of the protein generally will be retained when less than the majority of the residues of the complete or extracellular domain of the protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the amino terminus of the amino acid sequence of the Neutrokine-a shown in Figures 1A and 1B (SEQ ID NO:2), up to the glycine residue at position 191 (Gly-191 residue from the amino terminus), and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues n^1-285 of SEQ ID NO:2, where n^1 is an integer in the range of the amino acid position of amino acid residues 2-190 of the amino acid sequence in SEQ ID NO:2. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues 2-285, 3-285, 4-285, 5-285, 6-285, 7-285, 8-285, 9-285, 10-285, 11-285, 12-285, 13-285, 14-285, 15-285, 16-285, 17-285, 18-285, 19-285,

20-285, 21-285, 22-285, 23-285, 24-285, 25-285, 26-285, 27-285, 28-285, 29-285, 30-285, 31-285, 32-285, 33-285, 34-285, 35-285, 36-285, 37-285, 38-285, 39-285, 40-285, 41-285, 42-285, 43-285, 44-285, 45-285, 46-285, 47-285, 48-285, 49-285, 50-285, 51-285, 52-285, 53-285, 54-285, 55-285, 56-285, 57-285, 58-285, 59-285, 60-285, 61-285, 62-285, 63-285, 64-285, 65-285, 66-285, 67-285, 68-285, 69-285, 70-285, 71-285, 72-285, 73-285, 74-285, 75-285, 76-285, 77-285, 78-285, 79-285, 80-285, 81-285, 82-285, 83-285, 84-285, 85-285, 86-285, 87-285, 88-285, 89-285, 90-285, 91-285, 92-285, 93-285, 94-285, 95-285, 96-285, 97-285, 98-285, 99-285, 100-285, 101-285, 102-285, 103-285, 104-285, 105-285, 106-285, 107-285, 108-285, 109-285, 110-285, 111-285, 112-285, 113-285, 114-285, 115-285, 116-285, 117-285, 118-285, 119-285, 120-285, 121-285, 122-285, 123-285, 124-285, 125-285, 126-285, 127-285, 128-285, 129-285, 130-285, 131-285, 132-285, 133-285, 134-285, 135-285, 136-285, 137-285, 138-285, 139-285, 140-285, 141-285, 142-285, 143-285, 144-285, 145-285, 146-285, 147-285, 148-285, 149-285, 150-285, 151-285, 152-285, 153-285, 154-285, 155-285, 156-285, 157-285, 158-285, 159-285, 160-285, 161-285, 162-285, 163-285, 164-285, 165-285, 166-285, 167-285, 168-285, 169-285, 170-285, 171-285, 172-285, 173-285, 174-285, 175-285, 176-285, 177-285, 178-285, 179-285, 180-285, 181-285, 182-285, 183-285, 184-285, 185-285, 186-285, 187-285, 188-285, 189-285, and 190-285 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, Interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., *J. Biotechnology* 7:199-216 (1988). Since the present protein is a member of the TNF polypeptide family, deletions of C-terminal amino acids up to the leucine residue at position 284 are expected to retain most if not all biological activity such as, for example, ligand binding, the ability to stimulate lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and modulation of cell replication. Polypeptides having deletions of up to about 10 additional C-terminal residues (i.e., up to the glycine residue at position 274) also may retain some activity such as receptor binding, although such polypeptides would lack a portion of the conserved TNF domain which extends to about Leu-284 of SEQ ID NO:2. However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature protein

20-285, 21-285, 22-285, 23-285, 24-285, 25-285, 26-285, 27-285, 28-285, 29-285, 30-285, 31-285, 32-285, 33-285, 34-285, 35-285, 36-285, 37-285, 38-285, 39-285, 40-285, 41-285, 42-285, 43-285, 44-285, 45-285, 46-285, 47-285, 48-285, 49-285, 50-285, 51-285, 52-285, 53-285, 54-285, 55-285, 56-285, 57-285, 58-285, 59-285, 60-285, 61-285, 62-285, 63-285, 64-285, 65-285, 66-285, 67-285, 68-285, 69-285, 70-285, 71-285, 72-285, 73-285, 74-285, 75-285, 76-285, 77-285, 78-285, 79-285, 80-285, 81-285, 82-285, 83-285, 84-285, 85-285, 86-285, 87-285, 88-285, 89-285, 90-285, 91-285, 92-285, 93-285, 94-285, 95-285, 96-285, 97-285, 98-285, 99-285, 100-285, 101-285, 102-285, 103-285, 104-285, 105-285, 106-285, 107-285, 108-285, 109-285, 110-285, 111-285, 112-285, 113-285, 114-285, 115-285, 116-285, 117-285, 118-285, 119-285, 120-285, 121-285, 122-285, 123-285, 124-285, 125-285, 126-285, 127-285, 128-285, 129-285, 130-285, 131-285, 132-285, 133-285, 134-285, 135-285, 136-285, 137-285, 138-285, 139-285, 140-285, 141-285, 142-285, 143-285, 144-285, 145-285, 146-285, 147-285, 148-285, 149-285, 150-285, 151-285, 152-285, 153-285, 154-285, 155-285, 156-285, 157-285, 158-285, 159-285, 160-285, 161-285, 162-285, 163-285, 164-285, 165-285, 166-285, 167-285, 168-285, 169-285, 170-285, 171-285, 172-285, 173-285, 174-285, 175-285, 176-285, 177-285, 178-285, 179-285, 180-285, 181-285, 182-285, 183-285, 184-285, 185-285, 186-285, 187-285, 188-285, 189-285, and 190-285 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, Interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., *J. Biotechnology* 7:199-216 (1988). Since the present protein is a member of the TNF polypeptide family, deletions of C-terminal amino acids up to the leucine residue at position 284 are expected to retain most if not all biological activity such as, for example, ligand binding, the ability to stimulate lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and modulation of cell replication. Polypeptides having deletions of up to about 10 additional C-terminal residues (i.e., up to the glycine residue at position 274) also may retain some activity such as receptor binding, although such polypeptides would lack a portion of the conserved TNF domain which extends to about Leu-284 of SEQ ID NO:2. However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature protein

generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the Neutrokine-α shown in Figures 1A and 1B (SEQ ID NO:2), up to the glycine residue at position 274 (Gly-274) and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m^1 of the amino acid sequence in SEQ ID NO:2, where m^1 is any integer in the range of the amino acid position of amino acid residues 274-284 in SEQ ID NO:2. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues 1-274, 1-275, 1-276, 1-277, 1-278, 1-279, 1-280, 1-281, 1-282, 1-283 and 1-284 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also provided are polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues n^1-m^1 of SEQ ID NO:2, where n^1 and m^1 are integers as defined above. Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of the complete Neutrokine-α amino acid sequence encoded by the deposited cDNA clone contained in ATCC Accession No. 97768 where this portion excludes from 1 to 190 amino acids from the amino terminus or from 1 to 11 amino acids from the C-terminus of the complete amino acid sequence (or any combination of these N-terminal and C-terminal deletions) encoded by the cDNA clone in the deposited clone. Polynucleotides encoding all of the above deletion polypeptides are encompassed by the invention.

In specific embodiments, the following N- and/or C-terminally deleted polypeptide fragments of Neutrokine-a and/or Neutrokine-aSV are preferred: amino acid residues Ala-71 through Leu-285, amino acid residues Ala-81 through Leu-285, amino acid residues Leu-112 through Leu-285, amino acid residues Ala-134 through Leu-285, amino acid residues Leu-147 through Leu-285, and amino acid residues Gly-161 through Leu-285 of SEQ ID NO:2.

Furthermore, since the predicted extracellular domain of the Neutrokine-a polypeptides of the invention may itself elicit biological activity, deletions of N- and C-terminal amino acid residues from the predicted

extracellular region of the polypeptide (spanning positions Gln-73 to Leu-285 of SEQ ID NO:2) may retain some biological activity such as, for example, ligand binding, stimulation of lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and modulation of cell replication or modulation of target cell activities. However, even if deletion of one or more amino acids from the N-terminus of the predicted extracellular domain of a Neutrokine-a polypeptide results in modification of loss of one or more biological functions of the polypeptide, other functional activities may still be retained. Thus, the ability of the shortened polypeptides to induce and/or bind to antibodies which recognize the complete or mature or extracellular domains of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature or extracellular domains of the polypeptides are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of Neutrokine-a shown in SEQ ID NO:2, up to the glycine residue at position number 280, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n^2-285 of SEQ ID NO:2, where n^2 is an integer in the range of the amino acid position of amino acid residues 73-280 in SEQ ID NO:2, and 73 is the position of the first residue from the N-terminus of the predicted extracellular domain of the Neutrokine-a polypeptide (disclosed in SEQ ID NO:2).

More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of Q-73 to L-285; G-74 to L-285; D-75 to L-285; L-76 to L-285; A-77 to L-285; S-78 to L-285; L-79 to L-285; R-80 to L-285; A-81 to L-285; E-82 to L-285; L-83 to L-285; Q-84 to L-285; G-85 to L-285; H-86 to L-285; H-87 to L-285; A-88 to L-285; E-89 to L-285; K-90 to L-285; L-91 to L-285; P-92 to L-285; A-93 to L-285; G-94 to L-285; A-95 to L-285; G-96 to L-285; A-97 to L-285; P-98 to L-285; K-99 to L-285; A-100 to L-285; G-101 to L-285; L-102 to L-285; E-103 to L-285; E-104 to L-285; A-105 to L-285; P-106 to L-285; A-107 to L-285; V-108 to L-285; T-109 to L-285; A-110 to L-285; G-111 to L-285; L-112 to L-285; K-113 to L-285; I-114 to L-285; F-115 to L-285; E-116 to L-285; P-117 to L-285; P-118 to L-285; A-119 to L-285; P-120 to L-285; G-121 to L-285; E-122 to L-285;

G-123 to L-285; N-124 to L-285; S-125 to L-285; S-126 to L-285; Q-127 to L-285; N-128 to L-285; S-129 to L-285; R-130 to L-285; N-131 to L-285; K-132 to L-285; R-133 to L-285; A-134 to L-285; V-135 to L-285; Q-136 to L-285; G-137 to L-285; P-138 to L-285; E-139 to L-285; E-140 to L-285; T-141 to L-285; V-142 to L-285; T-143 to L-285; Q-144 to L-285; D-145 to L-285; C-146 to L-285; L-147 to L-285; Q-148 to L-285; L-149 to L-285; I-150 to L-285; A-151 to L-285; D-152 to L-285; S-153 to L-285; E-154 to L-285; T-155 to L-285; P-156 to L-285; T-157 to L-285; I-158 to L-285; Q-159 to L-285; K-160 to L-285; G-161 to L-285; S-162 to L-285; Y-163 to L-285; T-164 to L-285; F-165 to L-285; V-166 to L-285; P-167 to L-285; W-168 to L-285; L-169 to L-285; L-170 to L-285; S-171 to L-285; F-172 to L-285; K-173 to L-285; R-174 to L-285; G-175 to L-285; S-176 to L-285; A-177 to L-285; L-178 to L-285; E-179 to L-285; E-180 to L-285; K-181 to L-285; E-182 to L-285; N-183 to L-285; K-184 to L-285; I-185 to L-285; L-186 to L-285; V-187 to L-285; K-188 to L-285; E-189 to L-285; T-190 to L-285; G-191 to L-285; Y-192 to L-285; F-193 to L-285; F-194 to L-285; I-195 to L-285; Y-196 to L-285; G-197 to L-285; Q-198 to L-285; V-199 to L-285; L-200 to L-285; Y-201 to L-285; T-202 to L-285; D-203 to L-285; K-204 to L-285; T-205 to L-285; Y-206 to L-285; A-207 to L-285; M-208 to L-285; G-209 to L-285; H-210 to L-285; L-211 to L-285; I-212 to L-285; Q-213 to L-285; R-214 to L-285; K-215 to L-285; K-216 to L-285; V-217 to L-285; H-218 to L-285; V-219 to L-285; F-220 to L-285; G-221 to L-285; D-222 to L-285; E-223 to L-285; L-224 to L-285; S-225 to L-285; L-226 to L-285; V-227 to L-285; T-228 to L-285; L-229 to L-285; F-230 to L-285; R-231 to L-285; C-232 to L-285; I-233 to L-285; Q-234 to L-285; N-235 to L-285; M-236 to L-285; P-237 to L-285; E-238 to L-285; T-239 to L-285; L-240 to L-285; P-241 to L-285; N-242 to L-285; N-243 to L-285; S-244 to L-285; C-245 to L-285; Y-246 to L-285; S-247 to L-285; A-248 to L-285; G-249 to L-285; I-250 to L-285; A-251 to L-285; K-252 to L-285; L-253 to L-285; E-254 to L-285; E-255 to L-285; G-256 to L-285; D-257 to L-285; E-258 to L-285; L-259 to L-285; Q-260 to L-285; L-261 to L-285; A-262 to L-285; I-263 to L-285; P-264 to L-285; R-265 to L-285; E-266 to L-285; N-267 to L-285; A-268 to L-285; Q-269 to L-285; I-270 to L-285; S-271 to L-285; L-272 to L-285; D-273 to L-285; G-274 to L-285; D-275 to L-285; V-276 to L-285; T-277 to L-285; F-278 to L-285; F-279 to L-285; and G-280 to L-285 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Similarly, deletions of C-terminal amino acid residues of the predicted extracellular domain of Neutrokine-a up to the leucine residue at position 79 of

SEQ ID NO:2 may retain some biological activity, such as, for example, ligand binding, stimulation of lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and modulation of cell replication or modulation of target cell activities. Polypeptides having further C-terminal deletions including Leu-79 of SEQ ID NO:2 would not be expected to retain biological activities.

However, even if deletion of one or more amino acids from the C-terminus of a polypeptide results in modification of loss of one or more biological functions of the polypeptide, other functional activities may still be retained. Thus, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete, mature or extracellular forms of the polypeptide generally will be retained when less than the majority of the residues of the complete, mature or extracellular forms of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of the predicted extracellular domain retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the predicted extracellular domain of Neutrokine-a shown in SEQ ID NO:2, up to the leucine residue at position 79 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 73-m^2 of the amino acid sequence in SEQ ID NO:2, where m^2 is any integer in the range of the amino acid position of amino acid residues 79 to 285 in the amino acid sequence in SEQ ID NO:2, and residue 78 is the position of the first residue at the C-terminus of the predicted extracellular domain of the Neutrokine-a polypeptide (disclosed in SEQ ID NO:2).

More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues Q-73 to Leu-285; Q-73 to L-284; Q-73 to K-283; Q-73 to L-282; Q-73 to A-281; Q-73 to G-280; Q-73 to F-279; Q-73 to F-278; Q-73 to T-277; Q-73 to V-276; Q-73 to D-275; Q-73 to G-274; Q-73 to D-273; Q-73 to L-272; Q-73 to S-271; Q-73 to I-270; Q-73 to Q-269; Q-73 to A-268; Q-73 to N-267; Q-73 to E-266; Q-73 to R-265; Q-73 to P-264; Q-73 to I-263; Q-73 to A-262; Q-73 to L-261; Q-73 to Q-260; Q-73 to L-259; Q-73 to E-258; Q-73 to D-257; Q-73 to G-256; Q-73 to E-255; Q-73 to E-254; Q-73 to L-253; Q-73 to K-252; Q-73 to A-251; Q-73 to I-250; Q-73 to G-249; Q-73 to A-248; Q-73 to S-247; Q-73 to Y-246; Q-73 to C-245; Q-73 to S-244; Q-73 to N-243; Q-73 to N-242; Q-73 to P-241; Q-73 to L-240; Q-73 to T-239; Q-73 to

E-238; Q-73 to P-237; Q-73 to M-236; Q-73 to N-235; Q-73 to Q-234; Q-73 to I-233; Q-73 to C-232; Q-73 to R-231; Q-73 to F-230; Q-73 to L-229; Q-73 to T-228; Q-73 to V-227; Q-73 to L-226; Q-73 to S-225; Q-73 to L-224; Q-73 to E-223; Q-73 to D-222; Q-73 to G-221; Q-73 to F-220; Q-73 to V-219; Q-73 to H-218; Q-73 to V-217; Q-73 to K-216; Q-73 to K-215; Q-73 to R-214; Q-73 to Q-213; Q-73 to I-212; Q-73 to L-211; Q-73 to H-210; Q-73 to G-209; Q-73 to M-208; Q-73 to A-207; Q-73 to Y-206; Q-73 to T-205; Q-73 to K-204; Q-73 to D-203; Q-73 to T-202; Q-73 to Y-201; Q-73 to L-200; Q-73 to V-199; Q-73 to Q-198; Q-73 to G-197; Q-73 to Y-196; Q-73 to I-195; Q-73 to F-194; Q-73 to F-193; Q-73 to Y-192; Q-73 to G-191; Q-73 to T-190; Q-73 to E-189; Q-73 to K-188; Q-73 to V-187; Q-73 to L-186; Q-73 to I-185; Q-73 to K-184; Q-73 to N-183; Q-73 to E-182; Q-73 to K-181; Q-73 to E-180; Q-73 to E-179; Q-73 to L-178; Q-73 to A-177; Q-73 to S-176; Q-73 to G-175; Q-73 to R-174; Q-73 to K-173; Q-73 to F-172; Q-73 to S-171; Q-73 to L-170; Q-73 to L-169; Q-73 to W-168; Q-73 to P-167; Q-73 to V-166; Q-73 to F-165; Q-73 to T-164; Q-73 to Y-163; Q-73 to S-162; Q-73 to G-161; Q-73 to K-160; Q-73 to Q-159; Q-73 to I-158; Q-73 to T-157; Q-73 to P-156; Q-73 to T-155; Q-73 to E-154; Q-73 to S-153; Q-73 to D-152; Q-73 to A-151; Q-73 to I-150; Q-73 to L-149; Q-73 to Q-148; Q-73 to L-147; Q-73 to C-146; Q-73 to D-145; Q-73 to Q-144; Q-73 to T-143; Q-73 to V-142; Q-73 to T-141; Q-73 to E-140; Q-73 to E-139; Q-73 to P-138; Q-73 to G-137; Q-73 to Q-136; Q-73 to V-135; Q-73 to A-134; Q-73 to R-133; Q-73 to K-132; Q-73 to N-131; Q-73 to R-130; Q-73 to S-129; Q-73 to N-128; Q-73 to Q-127; Q-73 to S-126; Q-73 to S-125; Q-73 to N-124; Q-73 to G-123; Q-73 to E-122; Q-73 to G-121; Q-73 to P-120; Q-73 to A-119; Q-73 to P-118; Q-73 to P-117; Q-73 to E-116; Q-73 to F-115; Q-73 to I-114; Q-73 to K-113; Q-73 to L-112; Q-73 to G-111; Q-73 to A-110; Q-73 to T-109; Q-73 to V-108; Q-73 to A-107; Q-73 to P-106; Q-73 to A-105; Q-73 to E-104; Q-73 to E-103; Q-73 to L-102; Q-73 to G-101; Q-73 to A-100; Q-73 to K-99; Q-73 to P-98; Q-73 to A-97; Q-73 to G-96; Q-73 to A-95; Q-73 to G-94; Q-73 to A-93; Q-73 to P-92; Q-73 to L-91; Q-73 to K-90; Q-73 to E-89; Q-73 to A-88; Q-73 to H-87; Q-73 to H-86; Q-73 to G-85; Q-73 to Q-84; Q-73 to L-83; Q-73 to E-82; Q-73 to A-81; Q-73 to R-80; and Q-73 to L-79 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of the predicted extracellular domain of Neutrokine-a, which may be described generally as having residues n^2-m^2 of SEQ ID NO:2 where n^2 and m^2 are integers as defined above.

In another embodiment, a nucleotide sequence encoding a polypeptide consisting of a portion of the extracellular domain of the Neutrokine-a amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession no. 97768, where this portion excludes from 1 to about 206 amino acids from the amino terminus of the extracellular domain of the amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession no. 97768, or from 1 to about 206 amino acids from the carboxy terminus of the extracellular domain of the amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession no. 97768, or any combination of the above amino terminal and carboxy terminal deletions, of the entire extracellular domain of the amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession no. 97768.

As mentioned above, even if deletion of one or more amino acids from the N-terminus of a polypeptide results in modification of loss of one or more functional activities (e.g., biological activity) of the polypeptide, other functions or biological activities may still be retained. Thus, the ability of a shortened Neutrokine-a mutein to induce and/or bind to antibodies which recognize the full-length or mature forms or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the full-length or mature or extracellular domain of the polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokine-a mutein with a large number of deleted N-terminal amino acid residues may retain some functional (e.g., biological or immunogenic) activities. In fact, peptides composed of as few as six Neutrokine-a amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the predicted full-length amino acid sequence of the Neutrokine-a shown in SEQ ID NO:2, up to the glycine residue at position number 280 of the sequence shown SEQ ID NO:2 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n^3-285 of the sequence shown in SEQ ID NO:2, where n^3 is an integer in the range of the amino acid position of amino acid residues 1 to 280 of the amino acid sequence in SEQ ID NO:2.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of D-2 to L-285; D-3 to L-285; S-4 to L-285; T-5 to L-285; E-6 to L-285; R-7 to L-285; E-8 to L-285; Q-9 to L-285; S-10 to L-285; R-11 to L-285; L-12 to L-285; T-13 to L-285; S-14 to L-285; C-15 to L-285; L-16 to L-285; K-17 to L-285; K-18 to L-285; R-19 to L-285; E-20 to L-285; E-21 to L-285; M-22 to L-285; K-23 to L-285; L-24 to L-285; K-25 to L-285; E-26 to L-285; C-27 to L-285; V-28 to L-285; S-29 to L-285; I-30 to L-285; L-31 to L-285; P-32 to L-285; R-33 to L-285; K-34 to L-285; E-35 to L-285; S-36 to L-285; P-37 to L-285; S-38 to L-285; V-39 to L-285; R-40 to L-285; S-41 to L-285; S-42 to L-285; K-43 to L-285; D-44 to L-285; G-45 to L-285; K-46 to L-285; L-47 to L-285; L-48 to L-285; A-49 to L-285; A-50 to L-285; T-51 to L-285; L-52 to L-285; L-53 to L-285; L-54 to L-285; A-55 to L-285; L-56 to L-285; L-57 to L-285; S-58 to L-285; C-59 to L-285; C-60 to L-285; L-61 to L-285; T-62 to L-285; V-63 to L-285; V-64 to L-285; S-65 to L-285; F-66 to L-285; Y-67 to L-285; Q-68 to L-285; V-69 to L-285; A-70 to L-285; A-71 to L-285; L-72 to L-285; Q-73 to L-285; G-74 to L-285; D-75 to L-285; L-76 to L-285; A-77 to L-285; S-78 to L-285; L-79 to L-285; R-80 to L-285; A-81 to L-285; E-82 to L-285; L-83 to L-285; Q-84 to L-285; G-85 to L-285; H-86 to L-285; H-87 to L-285; A-88 to L-285; E-89 to L-285; K-90 to L-285; L-91 to L-285; P-92 to L-285; A-93 to L-285; G-94 to L-285; A-95 to L-285; G-96 to L-285; A-97 to L-285; P-98 to L-285; K-99 to L-285; A-100 to L-285; G-101 to L-285; L-102 to L-285; E-103 to L-285; E-104 to L-285; A-105 to L-285; P-106 to L-285; A-107 to L-285; V-108 to L-285; T-109 to L-285; A-110 to L-285; G-111 to L-285; L-112 to L-285; K-113 to L-285; I-114 to L-285; F-115 to L-285; E-116 to L-285; P-117 to L-285; P-118 to L-285; A-119 to L-285; P-120 to L-285; G-121 to L-285; E-122 to L-285; G-123 to L-285; N-124 to L-285; S-125 to L-285; S-126 to L-285; Q-127 to L-285; N-128 to L-285; S-129 to L-285; R-130 to L-285; N-131 to L-285; K-132 to L-285; R-133 to L-285; A-134 to L-285; V-135 to L-285; Q-136 to L-285; G-137 to L-285; P-138 to L-285; E-139 to L-285; E-140 to L-285; T-141 to L-285; V-142 to L-285; T-143 to L-285; Q-144 to L-285; D-145 to L-285; C-146 to L-285; L-147 to L-285; Q-148 to L-285; L-149 to L-285; I-150 to L-285; A-151 to L-285; D-152 to L-285; S-153 to L-285; E-154 to L-285; T-155 to L-285; P-156 to L-285; T-157 to L-285; I-158 to L-285; Q-159 to L-285; K-160 to L-285; G-161 to L-285; S-162 to L-285; Y-163 to L-285; T-164 to L-285; F-165 to L-285; V-166 to L-285; P-167 to L-285; W-168 to L-285; L-169 to L-285; L-170 to L-285; S-171 to L-285; F-172 to L-285; K-173 to L-285; R-174 to L-285; G-175 to L-285; S-176 to L-285; A-177 to L-285; L-178 to

L-285; E-179 to L-285; E-180 to L-285; K-181 to L-285; E-182 to L-285; N-183 to L-285; K-184 to L-285; I-185 to L-285; L-186 to L-285; V-187 to L-285; K-188 to L-285; E-189 to L-285; T-190 to L-285; G-191 to L-285; Y-192 to L-285; F-193 to L-285; F-194 to L-285; I-195 to L-285; Y-196 to L-285; G-197 to L-285; Q-198 to L-285; V-199 to L-285; L-200 to L-285; Y-201 to L-285; T-202 to L 285; D-203 to L-285; K-204 to L-285; T-205 to L-285; Y-206 to L-285; A-207 to L-285; M-208 to L-285; G-209 to L-285; H-210 to L-285; L-211 to L-285; I-212 to L-285; Q-213 to L-285; R-214 to L-285; K-215 to L-285; K-216 to L-285; V-217 to L-285; H-218 to L-285; V-219 to L-285; F-220 to L-285; G-221 to L-285; D-222 to L-285; E-223 to L-285; L-224 to L-285; S-225 to L-285; L-226 to L-285; V-227 to L-285; T-228 to L-285; L-229 to L-285; F-230 to L-285; R-231 to L-285; C-232 to L-285; I-233 to L-285; Q-234 to L-285; N-235 to L-285; M-236 to L-285; P-237 to L-285; E-238 to L-285; T-239 to L-285; L-240 to L-285; P-241 to L-285; N-242 to L-285; N-243 to L-285; S-244 to L-285; C-245 to L-285; Y-246 to L-285; S-247 to L-285; A-248 to L-285; G-249 to L-285; I-250 to L-285; A-251 to L-285; K-252 to L-285; L-253 to L-285; E-254 to L-285; E-255 to L-285; G-256 to L-285; D-257 to L-285; E-258 to L-285; L-259 to L-285; Q-260 to L-285; L-261 to L-285; A-262 to L-285; I-263 to L-285; P-264 to L-285; R-265 to L-285; E-266 to L-285; N-267 to L-285; A-268 to L-285; Q-269 to L-285; I-270 to L-285; S-271 to L-285; L-272 to L-285; D-273 to L-285; G-274 to L-285; D-275 to L-285; V-276 to L-285; T-277 to L-285; F-278 to L-285; F-279 to L-285; and G-280 to L-285 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more functional activities (e.g., biological activity) of the protein, other functional activities may still be retained. Thus, the ability of a shortened Neutrokine-α mutein to induce and/or bind to antibodies which recognize the complete or mature form or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature form or the extracellular domain of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokine-α mutein with a large number of deleted C-terminal amino acid residues may retain some functional (e.g., biological or

immunogenic) activities. In fact, peptides composed of as few as six Neutrokine-a amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides in another embodiment, polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the Neutrokine-a shown in SEQ ID NO:2, up to the glutamic acid residue at position number 6, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1-m^3 of SEQ ID NO:2, where m^3 is an integer in the range of the amino acid position of amino acid residues 6-284 of the amino acid sequence in SEQ ID NO:2.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues M-1 to L-284; M-1 to K-283; M-1 to L-282; M-1 to A-281; M-1 to G-280; M-1 to F-279; M-1 to F-278; M-1 to T-277; M-1 to V-276; M-1 to D-275; M-1 to G-274; M-1 to D-273; M-1 to L-272; M-1 to S-271; M-1 to I-270; M-1 to Q-269; M-1 to A-268; M-1 to N-267; M-1 to E-266; M-1 to R-265; M-1 to P-264; M-1 to I-263; M-1 to A-262; M-1 to L-261; M-1 to Q-260; M-1 to L-259; M-1 to E-258; M-1 to D-257; M-1 to G-256; M-1 to E-255; M-1 to E-254; M-1 to L-253; M-1 to K-252; M-1 to A-251; M-1 to I-250; M-1 to G-249; M-1 to A-248; M-1 to S-247; M-1 to Y-246; M-1 to C-245; M-1 to S-244; M-1 to N-243; M-1 to N-242; M-1 to P-241; M-1 to L-240; M-1 to T-239; M-1 to E-238; M-1 to P-237; M-1 to M-236; M-1 to N-235; M-1 to Q-234; M-1 to I-233; M-1 to C-232; M-1 to R-231; M-1 to F-230; M-1 to L-229; M-1 to T-228; M-1 to V-227; M-1 to L-226; M-1 to S-225; M-1 to L-224; M-1 to E-223; M-1 to D-222; M-1 to G-221; M-1 to F-220; M-1 to V-219; M-1 to H-218; M-1 to V-217; M-1 to K-216; M-1 to K-215; M-1 to R-214; M-1 to Q-213; M-1 to I-212; M-1 to L-211; M-1 to H-210; M-1 to G-209; M-1 to M-208; M-1 to A-207; M-1 to Y-206; M-1 to T-205; M-1 to K-204; M-1 to D-203; M-1 to T-202; M-1 to Y-201; M-1 to L-200; M-1 to V-199; M-1 to Q-198; M-1 to G-197; M-1 to Y-196; M-1 to I-195; M-1 to F-194; M-1 to F-193; M-1 to Y-192; M-1 to G-191; M-1 to T-190; M-1 to E-189; M-1 to K-188; M-1 to V-187; M-1 to L-186; M-1 to I-185; M-1 to K-184; M-1 to N-183; M-1 to E-182; M-1 to K-181; M-1 to E-180; M-1 to E-179; M-1 to L-178; M-1 to A-177; M-1 to S-176; M-1 to G-175; M-1 to R-174; M-1 to K-173; M-1 to F-172; M-1 to S-171; M-1 to L-170; M-1 to L-169; M-1 to W-168; M-1 to P-167; M-1 to V-166; M-1 to F-165; M-1 to T-164; M-1 to Y-163; M-1 to S-162; M-1 to G-161; M-1 to K-160; M-1 to Q-159; M-1 to I-158; M-1 to T-157; M-1 to P-156; M-1 to

T-155; M-1 to E-154; M-1 to S-153; M-1 to D-152; M-1 to A-151; M-1 to I-150; M-1 to L-149; M-1 to Q-148; M-1 to L-147; M-1 to C-146; M-1 to D-145; M-1 to Q-144; M-1 to T-143; M-1 to V-142; M-1 to T-141; M-1 to E-140; M-1 to E-139; M-1 to P-138; M-1 to G-137; M-1 to Q-136; M-1 to V-135; M-1 to A-134; M-1 to R-133; M-1 to K-132; M-1 to N-131; M-1 to R-130; M-1 to S-129; M-1 to N-128; M-1 to Q-127; M-1 to S-126; M-1 to S-125; M-1 to N-124; M-1 to G-123; M-1 to E-122; M-1 to G-121; M-1 to P-120; M-1 to A-119; M-1 to P-118; M-1 to P-117; M-1 to E-116; M-1 to F-115; M-1 to I-114; M-1 to K-113; M-1 to L-112; M-1 to G-111; M-1 to A-110; M-1 to T-109; M-1 to V-108; M-1 to A-107; M-1 to P-106; M-1 to A-105; M-1 to E-104; M-1 to E-103; M-1 to L-102; M-1 to G-101; M-1 to A-100; M-1 to K-99; M-1 to P-98; M-1 to A-97; M-1 to G-96; M-1 to A-95; M-1 to G-94; M-1 to A-93; M-1 to P-92; M-1 to L-91; M-1 to K-90; M-1 to E-89; M-1 to A-88; M-1 to H-87; M-1 to H-86; M-1 to G-85; M-1 to Q-84; M-1 to L-83; M-1 to E-82; M-1 to A-81; M-1 to R-80; M-1 to L-79; M-1 to S-78; M-1 to A-77; M-1 to L-76; M-1 to D-75; M-1 to G-74; M-1 to Q-73; M-1 to L-72; M-1 to A-71; M-1 to A-70; M-1 to V-69; M-1 to Q-68; M-1 to Y-67; M-1 to F-66; M-1 to S-65; M-1 to V-64; M-1 to V-63; M-1 to T-62; M-1 to L-61; M-1 to C-60; M-1 to C-59; M-1 to S-58; M-1 to L-57; M-1 to L-56; M-1 to A-55; M-1 to L-54; M-1 to L-53; M-1 to L-52; M-1 to T-51; M-1 to A-50; M-1 to A-49; M-1 to L-48; M-1 to L-47; M-1 to K-46; M-1 to G-45; M-1 to D-44; M-1 to K-43; M-1 to S-42; M-1 to S-41; M-1 to R-40; M-1 to V-39; M-1 to S-38; M-1 to P-37; M-1 to S-36; M-1 to E-35; M-1 to K-34; M-1 to R-33; M-1 to P-32; M-1 to L-31; M-1 to I-30; M-1 to S-29; M-1 to V-28; M-1 to C-27; M-1 to E-26; M-1 to K-25; M-1 to L-24; M-1 to K-23; M-1 to M-22; M-1 to E-21; M-1 to E-20; M-1 to R-19; M-1 to K-18; M-1 to K-17; M-1 to L-16; M-1 to C-15; M-1 to S-14; M-1 to T-13; M-1 to L-12; M-1 to R-11; M-1 to S-10; M-1 to Q-9; M-1 to E-8; M-1 to R-7; and M-1 to E-6 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a Neutrokine-a polypeptide, which may be described generally as having residues n^3-m^3 of SEQ ID NO:2, where n^3 and m^3 are integers as defined above.

Furthermore, since the predicted extracellular domain of the Neutrokine-aSV polypeptides of the invention may itself elicit functional activity (e.g., biological activity), deletions of N- and C-terminal amino acid residues from the predicted extracellular region of the polypeptide at positions Gln-73 to Leu-266 of SEQ ID NO:19 may retain some functional activity, such as, for

example, ligand binding, to stimulation of lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, modulation of cell replication, modulation of target cell activities and/or immunogenicity. However, even if deletion of one or more amino acids from the N-terminus of the predicted extracellular domain of a Neutrokine-aSV polypeptide results in modification of loss of one or more functional activities of the polypeptide, other functional activities may still be retained. Thus, the ability of the shortened polypeptides to induce and/or bind to antibodies which recognize the complete or mature or extracellular domains of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature or extracellular domains of the polypeptides are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of Neutrokine-aSV shown in SEQ ID NO:19, up to the glycine residue at position number 261, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n^4-266 of SEQ ID NO:19, where n^4 is an integer in the range of the amino acid position of amino acid residues 73-261 of the amino acid sequence in SEQ ID NO:19, and 261 is the position of the first residue from the N-terminus of the predicted extracellular domain Neutrokine-aSV polypeptide (shown in SEQ ID NO:19).

More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of Q-73 to L-266; G-74 to L-266; D-75 to L-266; L-76 to L-266; A-77 to L-266; S-78 to L-266; L-79 to L-266; R-80 to L-266; A-81 to L-266; E-82 to L-266; L-83 to L-266; Q-84 to L-266; G-85 to L-266; H-86 to L-266; H-87 to L-266; A-88 to L-266; E-89 to L-266; K-90 to L-266; L-91 to L-266; P-92 to L-266; A-93 to L-266; G-94 to L-266; A-95 to L-266; G-96 to L-266; A-97 to L-266; P-98 to L-266; K-99 to L-266; A-100 to L-266; G-101 to L-266; L-102 to L-266; E-103 to L-266; E-104 to L-266; A-105 to L-266; P-106 to L-266; A-107 to L-266; V-108 to L-266; T-109 to L-266; A-110 to L-266; G-111 to L-266; L-112 to L-266; K-113 to L-266; I-114 to L-266; F-115 to L-266; E-116 to L-266; P-117 to L-266; P-118 to L-266; A-119 to L-266; P-120 to L-266; G-121 to L-266; E-122 to L-266; G-123 to L-266; N-124 to L-266; S-125 to L-266; S-126 to L-266; Q-127 to

L-266; N-128 to L-266; S-129 to L-266; R-130 to L-266; N-131 to L-266; K-132 to L-266; R-133 to L-266; A-134 to L-266; V-135 to L-266; Q-136 to L-266; G-137 to L-266; P-138 to L-266; E-139 to L-266; E-140 to L-266; T-141 to L-266; G-142 to L-266; S-143 to L-266; Y-144 to L-266; T-145 to L-266; F-146 to L-266; V-147 to L-266; P-148 to L-266; W-149 to L-266; L-150 to L-266; L-151 to L-266; S-152 to L-266; F-153 to L-266; K-154 to L-266; R-155 to L-266; G-156 to L-266; S-157 to L-266; A-158 to L-266; L-159 to L-266; E-160 to L-266; E-161 to L-266; K-162 to L-266; E-163 to L-266; N-164 to L-266; K-165 to L-266; I-166 to L-266; L-167 to L-266; V-168 to L-266; K-169 to L-266; E-170 to L-266; T-171 to L-266; G-172 to L-266; Y-173 to L-266; F-174 to L-266; F-175 to L-266; I-176 to L-266; Y-177 to L-266; G-178 to L-266; Q-179 to L-266; V-180 to L-266; L-181 to L-266; Y-182 to L-266; T-183 to L-266; D-184 to L-266; K-185 to L-266; T-186 to L-266; Y-187 to L-266; A-188 to L-266; M-189 to L-266; G-190 to L-266; H-191 to L-266; L-192 to L-266; I-193 to L-266; Q-194 to L-266; R-195 to L-266; K-196 to L-266; K-197 to L-266; V-198 to L-266; H-199 to L-266; V-200 to L-266; F-201 to L-266; G-202 to L-266; D-203 to L-266; E-204 to L-266; L-205 to L-266; S-206 to L-266; L-207 to L-266; V-208 to L-266; T-209 to L-266; L-210 to L-266; F-211 to L-266; R-212 to L-266; C-213 to L-266; I-214 to L-266; Q-215 to L-266; N-216 to L-266; M-217 to L-266; P-218 to L-266; E-219 to L-266; T-220 to L-266; L-221 to L-266; P-222 to L-266; N-223 to L-266; N-224 to L-266; S-225 to L-266; C-226 to L-266; Y-227 to L-266; S-228 to L-266; A-229 to L-266; G-230 to L-266; I-231 to L-266; A-232 to L-266; K-233 to L-266; L-234 to L-266; E-235 to L-266; E-236 to L-266; G-237 to L-266; D-238 to L-266; E-239 to L-266; L-240 to L-266; Q-241 to L-266; L-242 to L-266; A-243 to L-266; I-244 to L-266; P-245 to L-266; R-246 to L-266; E-247 to L-266; N-248 to L-266; A-249 to L-266; Q-250 to L-266; I-251 to L-266; S-252 to L-266; L-253 to L-266; D-254 to L-266; G-255 to L-266; D-256 to L-266; V-257 to L-266; T-258 to L-266; F-259 to L-266; F-260 to L-266; and G-261 to L-266 of SEQ ID NO:19. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Similarly, deletions of C-terminal amino acid residues of the predicted extracellular domain of Neutrokine-αSV up to the leucine residue at position 79 of SEQ ID NO:19 may retain some functional activity, such as, for example, ligand binding, the ability to stimulate lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, modulation of cell replication, modulation of target cell activities and/or immunogenicity. Polypeptides having further

C-terminal deletions including Leu-79 of SEQ ID NO:19 would not be expected to retain biological activities.

However, even if deletion of one or more amino acids from the C-terminus of a polypeptide results in modification of loss of one or more functional activities (e.g., biological activity) of the polypeptide, other functional activities may still be retained. Thus, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete, mature or extracellular forms of the polypeptide generally will be retained when less than the majority of the residues of the complete, mature or extracellular forms of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of the predicted extracellular domain retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the predicted extracellular domain of Neutrokine-αSV shown in SEQ ID NO:19, up to the leucine residue at position 79 of SEQ ID NO:19, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 73-m^4 of the amino acid sequence in SEQ ID NO:19, where m^4 is any integer in the range of the amino acid position of amino acid residues 79-266 of the amino acid sequence in SEQ ID NO:19.

More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues Q-73 to L-265; Q-73 to K-264; Q-73 to L-263; Q-73 to A-262; Q-73 to G-261; Q-73 to F-260; Q-73 to F-259; Q-73 to T-258; Q-73 to V-257; Q-73 to D-256; Q-73 to G-255; Q-73 to D-254; Q-73 to L-253; Q-73 to S-252; Q-73 to I-251; Q-73 to Q-250; Q-73 to A-249; Q-73 to N-248; Q-73 to E-247; Q-73 to R-246; Q-73 to P-245; Q-73 to I-244; Q-73 to A-243; Q-73 to L-242; Q-73 to Q-241; Q-73 to L-240; Q-73 to E-239; Q-73 to D-238; Q-73 to G-237; Q-73 to E-236; Q-73 to E-235; Q-73 to L-234; Q-73 to K-233; Q-73 to A-232; Q-73 to I-231; Q-73 to G-230; Q-73 to A-229; Q-73 to S-228; Q-73 to Y-227; Q-73 to C-226; Q-73 to S-225; Q-73 to N-224; Q-73 to N-223; Q-73 to P-222; Q-73 to L-221; Q-73 to T-220; Q-73 to E-219; Q-73 to P-218; Q-73 to M-217; Q-73 to N-216; Q-73 to Q-215; Q-73 to I-214; Q-73 to C-213; Q-73 to R-212; Q-73 to F-211; Q-73 to L-210; Q-73 to T-209; Q-73 to V-208; Q-73 to L-207; Q-73 to S-206; Q-73 to L-205; Q-73 to E-204; Q-73 to D-203; Q-73 to G-202; Q-73 to F-201; Q-73 to V-200; Q-73 to H-199; Q-73 to V-198; Q-73 to K-197; Q-73 to K-196; Q-73 to R-195; Q-73 to Q-194; Q-73 to

I-193; Q-73 to L-192; Q-73 to H-191; Q-73 to G-190; Q-73 to Q-7389; Q-73 to A-188; Q-73 to Y-187; Q-73 to T-186; Q-73 to K-185; Q-73 to D-184; Q-73 to T-183; Q-73 to Y-182; Q-73 to L-181; Q-73 to V-180; Q-73 to Q-179; Q-73 to G-178; Q-73 to Y-177; Q-73 to I-176; Q-73 to F-175; Q-73 to F-174; Q-73 to Y-173; Q-73 to G-172; Q-73 to T-171; Q-73 to E-170; Q-73 to K-169; Q-73 to V-168; Q-73 to L-167; Q-73 to I-166; Q-73 to K-165; Q-73 to N-164; Q-73 to E-163; Q-73 to K-162; Q-73 to E-161; Q-73 to E-160; Q-73 to L-159; Q-73 to A-158; Q-73 to S-157; Q-73 to G-156; Q-73 to R-155; Q-73 to K-154; Q-73 to F-153; Q-73 to S-152; Q-73 to L-151; Q-73 to L-150; Q-73 to W-149; Q-73 to P-148; Q-73 to V-147; Q-73 to F-146; Q-73 to T-145; Q-73 to Y-144; Q-73 to S-143; Q-73 to G-142; Q-73 to T-141; Q-73 to E-140; Q-73 to E-139; Q-73 to P-138; Q-73 to G-137; Q-73 to Q-136; Q-73 to V-135; Q-73 to A-134; Q-73 to R-133; Q-73 to K-132; Q-73 to N-131; Q-73 to R-130; Q-73 to S-129; Q-73 to N-128; Q-73 to Q-127; Q-73 to S-126; Q-73 to S-125; Q-73 to N-124; Q-73 to G-123; Q-73 to E-122; Q-73 to G-121; Q-73 to P-120; Q-73 to A-119; Q-73 to P-118; Q-73 to P-117; Q-73 to E-116; Q-73 to F-115; Q-73 to I-114; Q-73 to K-113; Q-73 to L-112; Q-73 to G-111; Q-73 to A-110; Q-73 to T-109; Q-73 to V-108; Q-73 to A-107; Q-73 to P-106; Q-73 to A-105; Q-73 to E-104; Q-73 to E-103; Q-73 to L-102; Q-73 to G-101; Q-73 to A-100; Q-73 to K-99; Q-73 to P-98; Q-73 to A-97; Q-73 to G-96; Q-73 to A-95; Q-73 to G-94; Q-73 to A-93; Q-73 to P-92; Q-73 to L-91; Q-73 to K-90; Q-73 to E-89; Q-73 to A-88; Q-73 to H-87; Q-73 to H-86; Q-73 to G-85; Q-73 to Q-84; Q-73 to L-83; Q-73 to E-82; Q-73 to A-81; Q-73 to R-80; Q-73 to L-79; and Q-73 to S-78 of SEQ ID NO:19. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of the predicted extracellular domain of Neutrokine-aSV, which may be described generally as having residues n^4-m^4 of SEQ ID NO:19 where n^4 and m^4 are integers as defined above.

In another embodiment, a nucleotide sequence encoding a polypeptide consisting of a portion of the extracellular domain of the Neutrokine-aSV amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC Accession No. 203518, where this portion excludes from 1 to about 260 amino acids from the amino terminus of the extracellular domain of the amino acid sequence encoded by cDNA clone contained in the deposit having ATCC Accession No. 203518, or from 1 to about 187 amino acids from the carboxy terminus of the extracellular domain of the amino acid sequence encoded by cDNA clone contained in the deposit having ATCC Accession No. 203518, or

any combination of the above amino terminal and carboxy terminal deletions, of the entire extracellular domain of the amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC Accession No. 203518.

As mentioned above, even if deletion of one or more amino acids from the N-terminus of a polypeptide results in modification of loss of one or more functional activities (e.g., biological activity) of the polypeptide, other functional activities may still be retained. Thus, the ability of a shortened Neutrokine-αSV mutein to induce and/or bind to antibodies which recognize the full-length or mature forms or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the full-length or mature or extracellular domain of the polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokine-αSV mutein with a large number of deleted N-terminal amino acid residues may retain functional (e.g., immunogenic) activities. In fact, peptides composed of as few as six Neutrokine-αSV amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the predicted full-length amino acid sequence of the Neutrokine-αSV shown in SEQ ID NO:19, up to the glycine residue at position number 261 of the sequence shown SEQ ID NO:19 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n^5-266 of the sequence shown in SEQ ID NO:19, where n^5 is an integer in the range of the amino acid position of amino acid residues 1 to 261 of the amino acid sequence in SEQ ID NO:19.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of D-2 to L-266; D-3 to L-266; S-4 to L-266; T-5 to L-266; E-6 to L-266; R-7 to L-266; E-8 to L-266; Q-9 to L-266; S-10 to L-266; R-11 to L-266; L-12 to L-266; T-13 to L-266; S-14 to L-266; C-15 to L-266; L-16 to L-266; K-17 to L-266; K-18 to L-266; R-19 to L-266; E-20 to L-266; E-21 to L-266; M-22 to L-266; K-23 to L-266; L-24 to L-266; K-25 to L-266; E-26 to L-266; C-27 to L-266; V-28 to L-266; S-29 to L-266; I-30 to L-266; L-31 to L-266; P-32 to L-266; R-33 to L-266; K-34 to L-266; E-35 to L-266; S-36 to L-266; P-37 to L-266; S-38 to L-266; V-39 to L-266; R-40 to L-266; S-41 to L-266; S-42 to L-266; K-43 to L-266; D-44 to L-266; G-45 to L-266; K-46 to

L-266; L-47 to L-266; L-48 to L-266; A-49 to L-266; A-50 to L-266; T-51 to L-266; L-52 to L-266; L-53 to L-266; L-54 to L-266; A-55 to L-266; L-56 to L-266; L-57 to L-266; S-58 to L-266; C-59 to L-266; C-60 to L-266; L-61 to L-266; T-62 to L-266; V-63 to L-266; V-64 to L-266; S-65 to L-266; F-66 to L-266; Y-67 to L-266; Q-68 to L-266; V-69 to L-266; A-70 to L-266; A-71 to L-266; L-72 to L-266; Q-73 to L-266; G-74 to L-266; D-75 to L-266; L-76 to L-266; A-77 to L-266; S-78 to L-266; L-79 to L-266; R-80 to L-266; A-81 to L-266; E-82 to L-266; L-83 to L-266; Q-84 to L-266; G-85 to L-266; H-86 to L-266; H-87 to L-266; A-88 to L-266; E-89 to L-266; K-90 to L-266; L-91 to L-266; P-92 to L-266; A-93 to L-266; G-94 to L-266; A-95 to L-266; G-96 to L-266; A-97 to L-266; P-98 to L-266; K-99 to L-266; A-100 to L-266; G-101 to L-266; L-102 to L-266; E-103 to L-266; E-104 to L-266; A-105 to L-266; P-106 to L-266; A-107 to L-266; V-108 to L-266; T-109 to L-266; A-110 to L-266; G-111 to L-266; L-112 to L-266; K-113 to L-266; I-114 to L-266; F-115 to L-266; E-116 to L-266; P-117 to L-266; P-118 to L-266; A-119 to L-266; P-120 to L-266; G-121 to L-266; E-122 to L-266; G-123 to L-266; N-124 to L-266; S-125 to L-266; S-126 to L-266; Q-127 to L-266; N-128 to L-266; S-129 to L-266; R-130 to L-266; N-131 to L-266; K-132 to L-266; R-133 to L-266; A-134 to L-266; V-135 to L-266; Q-136 to L-266; G-137 to L-266; P-138 to L-266; E-139 to L-266; E-140 to L-266; T-141 to L-266; G-142 to L-266; S-143 to L-266; Y-144 to L-266; T-145 to L-266; F-146 to L-266; V-147 to L-266; P-148 to L-266; W-149 to L-266; L-150 to L-266; L-151 to L-266; S-152 to L-266; F-153 to L-266; K-154 to L-266; R-155 to L-266; G-156 to L-266; S-157 to L-266; A-158 to L-266; L-159 to L-266; E-160 to L-266; E-161 to L-266; K-162 to L-266; E-163 to L-266; N-164 to L-266; K-165 to L-266; I-166 to L-266; L-167 to L-266; V-168 to L-266; K-169 to L-266; E-170 to L-266; T-171 to L-266; G-172 to L-266; Y-173 to L-266; F-174 to L-266; F-175 to L-266; I-176 to L-266; Y-177 to L-266; G-178 to L-266; Q-179 to L-266; V-180 to L-266; L-181 to L-266; Y-182 to L-266; T-183 to L-266; D-184 to L-266; K-185 to L-266; T-186 to L-266; Y-187 to L-266; A-188 to L-266; M-189 to L-266; G-190 to L-266; H-191 to L-266; L-192 to L-266; I-193 to L-266; Q-194 to L-266; R-195 to L-266; K-196 to L-266; K-197 to L-266; V-198 to L-266; H-199 to L-266; V-200 to L-266; F-201 to L-266; G-202 to L-266; D-203 to L-266; E-204 to L-266; L-205 to L-266; S-206 to L-266; L-207 to L-266; V-208 to L-266; T-209 to L-266; L-210 to L-266; F-211 to L-266; R-212 to L-266; C-213 to L-266; I-214 to L-266; Q-215 to L-266; N-216 to L-266; M-217 to L-266; P-218 to L-266; E-219 to L-266; T-220 to L-266; L-221 to L-266; P-222 to L-266; N-223 to L-266; N-224 to L-266; S-225 to L-266; C-226 to L-266; Y-227 to L-266;

S-228 to L-266; A-229 to L-266; G-230 to L-266; I-231 to L-266; A-232 to L-266; K-233 to L-266; L-234 to L-266; E-235 to L-266; E-236 to L-266; G-237 to L-266; D-238 to L-266; E-239 to L-266; L-240 to L-266; Q-241 to L-266; L-242 to L-266; A-243 to L-266; I-244 to L-266; P-245 to L-266; R-246 to L-266; E-247 to L-266; N-248 to L-266; A-249 to L-266; Q-250 to L-266; I-251 to L-266; S-252 to L-266; L-253 to L-266; D-254 to L-266; G-255 to L-266; D-256 to L-266; V-257 to L-266; T-258 to L-266; F-259 to L-266; F-260 to L-266; and G-261 to L-266 of SEQ ID NO:19. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more functional activities (e.g., biological activities) of the protein, other functional activities may still be retained. Thus, the ability of a shortened Neutrokine-aSV mutein to induce and/or bind to antibodies which recognize the complete or mature form or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature form or the extracellular domain of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokine-aSV mutein with a large number of deleted C-terminal amino acid residues may retain some functional (e.g., immunogenic) activities. In fact, peptides composed of as few as six Neutrokine-aSV amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides in another embodiment, polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the Neutrokine-aSV shown in SEQ ID NO:19, up to the glutamic acid residue at position number 6, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1-m^5 of SEQ ID NO:19, where m^5 is an integer in the range of the amino acid position of amino acid residues 6 to 265 in the amino acid sequence of SEQ ID NO:19.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues M-1 to L-265; M-1 to K-264; M-1 to L-263; M-1 to A-262; M-1 to G-261; M-1 to F-260; M-1 to F-259; M-1 to T-258; M-1 to V-257; M-1 to D-256; M-1 to G-255; M-1 to D-254; M-1 to L-253; M-1 to S-252; M-1 to

I-251; M-1 to Q-250; M-1 to A-249; M-1 to N-248; M-1 to E-247; M-1 to R-246; M-1 to P-245; M-1 to I-244; M-1 to A-243; M-1 to L-242; M-1 to Q-241; M-1 to L-240; M-1 to E-239; M-1 to D-238; M-1 to G-237; M-1 to E-236; M-1 to E-235; M-1 to L-234; M-1 to K-233; M-1 to A-232; M-1 to I-231; M-1 to G-230; M-1 to A-229; M-1 to S-228; M-1 to Y-227; M-1 to C-226; M-1 to S-225; M-1 to N-224; M-1 to N-223; M-1 to P-222; M-1 to L-221; M-1 to T-220; M-1 to E-219; M-1 to P-218; M-1 to M-217; M-1 to N-216; M-1 to Q-215; M-1 to I-214; M-1 to C-213; M-1 to R-212; M-1 to F-211; M-1 to L-210; M-1 to T-209; M-1 to V-208; M-1 to L-207; M-1 to S-206; M-1 to L-205; M-1 to E-204; M-1 to D-203; M-1 to G-202; M-1 to F-201; M-1 to V-200; M-1 to H-199; M-1 to V-198; M-1 to K-197; M-1 to K-196; M-1 to R-195; M-1 to Q-194; M-1 to I-193; M-1 to L-192; M-1 to H-191; M-1 to G-190; M-1 to M-189; M-1 to A-188; M-1 to Y-187; M-1 to T-186; M-1 to K-185; M-1 to D-184; M-1 to T-183; M-1 to Y-182; M-1 to L-181; M-1 to V-180; M-1 to Q-179; M-1 to G-178; M-1 to Y-177; M-1 to I-176; M-1 to F-175; M-1 to F-174; M-1 to Y-173; M-1 to G-172; M-1 to T-171; M-1 to E-170; M-1 to K-169; M-1 to V-168; M-1 to L-167; M-1 to I-166; M-1 to K-165; M-1 to N-164; M-1 to E-163; M-1 to K-162; M-1 to E-161; M-1 to E-160; M-1 to L-159; M-1 to A-158; M-1 to S-157; M-1 to G-156; M-1 to R-155; M-1 to K-154; M-1 to F-153; M-1 to S-152; M-1 to L-151; M-1 to L-150; M-1 to W-149; M-1 to P-148; M-1 to V-147; M-1 to F-146; M-1 to T-145; M-1 to Y-144; M-1 to S-143; M-1 to G-142; M-1 to T-141; M-1 to E-140; M-1 to E-139; M-1 to P-138; M-1 to G-137; M-1 to Q-136; M-1 to V-135; M-1 to A-134; M-1 to R-133; M-1 to K-132; M-1 to N-131; M-1 to R-130; M-1 to S-129; M-1 to N-128; M-1 to Q-127; M-1 to S-126; M-1 to S-125; M-1 to N-124; M-1 to G-123; M-1 to E-122; M-1 to G-121; M-1 to P-120; M-1 to A-119; M-1 to P-118; M-1 to P-117; M-1 to E-116; M-1 to F-115; M-1 to I-114; M-1 to K-113; M-1 to L-112; M-1 to G-111; M-1 to A-110; M-1 to T-109; M-1 to V-108; M-1 to A-107; M-1 to P-106; M-1 to A-105; M-1 to E-104; M-1 to E-103; M-1 to L-102; M-1 to G-101; M-1 to A-100; M-1 to K-99; M-1 to P-98; M-1 to A-97; M-1 to G-96; M-1 to A-95; M-1 to G-94; M-1 to A-93; M-1 to P-92; M-1 to L-91; M-1 to K-90; M-1 to E-89; M-1 to A-88; M-1 to H-87; M-1 to H-86; M-1 to G-85; M-1 to Q-84; M-1 to L-83; M-1 to E-82; M-1 to A-81; M-1 to R-80; M-1 to L-79; M-1 to S-78; M-1 to A-77; M-1 to L-76; M-1 to D-75; M-1 to G-74; M-1 to Q-73; M-1 to L-72; M-1 to A-71; M-1 to A-70; M-1 to V-69; M-1 to Q-68; M-1 to Y-67; M-1 to F-66; M-1 to S-65; M-1 to V-64; M-1 to V-63; M-1 to T-62; M-1 to L-61; M-1 to C-60; M-1 to C-59; M-1 to S-58; M-1 to L-57; M-1 to L-56; M-1 to A-55; M-1 to L-54; M-1 to L-53; M-1 to L-52; M-1 to T-51; M-1

to A-50: M-1 to A-49; M-1 to L-48; M-1 to L-47; M-1 to K-46; M-1 to G-45; M-1 to D-44; M-1 to K-43; M-1 to S-42; M-1 to S-41; M-1 to R-40; M-1 to V-39; M-1 to S-38; M-1 to P-37; M-1 to S-36; M-1 to E-35; M-1 to K-34; M-1 to R-33; M-1 to P-32; M-1 to L-31; M-1 to I-30; M-1 to S-29; M-1 to V-28; M-1 to C-27; M-1 to E-26; M-1 to K-25; M-1 to L-24; M-1 to K-23; M-1 to M-22; M-1 to E-21; M-1 to E-20; M-1 to R-19; M-1 to K-18; M-1 to K-17; M-1 to L-16; M-1 to C-15; M-1 to S-14; M-1 to T-13; M-1 to L-12; M-1 to R-11; M-1 to S-10; M-1 to Q-9; M-1 to E-8; M-1 to R-7; and M-1 to E-6 of SEQ ID NO:19. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a Neutrokine-αSV polypeptide, which may be described generally as having residues n^5-m^5 of SEQ ID NO:19, where n^5 and m^5 are integers as defined above.

Other Mutants

It will be recognized by one of ordinary skill in the art that some amino acid sequences of the Neutrokine-α and Neutrokine-αSV polypeptides can be varied without significant effect of the structure or function of the polypeptide. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the polypeptide which determine activity.

Thus, the invention further includes variations of the Neutrokine-α polypeptide which show substantial Neutrokine-α polypeptide functional activity (e.g., biological activity) or which include regions of Neutrokine-α polypeptide such as the protein portions discussed below. The invention also includes variations of the Neutrokine-αSV polypeptide which show substantial Neutrokine-αSV polypeptide functional activity (e.g., biological activity) or which include regions of Neutrokine-αSV polypeptide such as the polypeptide portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247*:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to

introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie, J. U. *et al., supra*, and the references cited therein. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Thus, the fragment, derivative or analog of the polypeptide of Figures 1A and 1B (SEQ ID NO:2), or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the extracellular domain of the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the extracellular domain of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the extracellular domain of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein

Furthermore, the fragment, derivative or analog of the polypeptide of Figures 5A and 5B (SEQ ID NO:19), or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the extracellular domain of the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the

extracellular domain of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the extracellular domain of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein

Thus, the Neutrokine-α and/or Neutrokine-αSV polypeptides of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table II).

TABLE II. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the Neutrokine-α and/or Neutrokine-αSV polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for functional activity, such ligand binding and the ability to stimulate lymphocyte (e.g., B cell) as, for example, proliferation, differentiation, and/or activation.

Of special interest are substitutions of charged amino acids with other charged or neutral amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may

not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard *et al., Clin. Exp. Immunol.* 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland *et al., Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993).

Replacement of amino acids can also change the selectivity of the binding of a ligand to cell surface receptors. For example, Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-a to only one of the two known types of TNF receptors. Since Neutrokine-α and Neutrokine-αSV is members of the TNF polypeptide family, mutations similar to those in TNF-α are likely to have similar effects in Neutrokine-α and/or Neutrokine-αSV.

Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)). Since Neutrokine-α is a member of the TNF-related protein family, to modulate rather than completely eliminate functional activities (e.g., biological activities) of Neutrokine-α, preferably mutations are made in sequences encoding amino acids in the TNF conserved domain, i.e., in positions Gly-191 through Leu-284 of Figures 1A and 1B (SEQ ID NO:2), more preferably in residues within this region which are not conserved in all members of the TGF family. By making a specific mutation in Neutrokine-α in the position where such a conserved amino acid is typically found in related TNFs, Neutrokine-α will act as an antagonist, thus possessing activity for example, which inhibits lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation. Accordingly, polypeptides of the present invention include Neutrokine-α mutants. Such Neutrokine-α mutants are comprised of the full-length or preferably the extracellular domain of the Neutrokine-α amino acid sequence shown in Figures 1A and 1B (SEQ ID NO:2). Polynucleotides encode the above Neutrokine-α mutants are also encompassed by the invention.

Since Neutrokine-αSV is a member of the TNF-related protein family, to modulate rather than completely eliminate functional activities (e.g., biological activities) of Neutrokine-αSV, preferably mutations are made in sequences encoding amino acids in the TNF conserved domain, i.e., in positions Gly-172 through Leu-265 of Figures 5A and 5B (SEQ ID NO:19), more preferably in residues within this region which are not conserved in all members of the TGF family. By making a specific mutation in Neutrokine-αSV in the position where such a conserved amino acid is typically found in related TNFs, Neutrokine-αSV will act as an antagonist, thus possessing activity for

example, which inhibits lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation. Accordingly, polypeptides of the present invention include Neutrokine-αSV mutants. Such Neutrokine-αSV mutants are comprised of the full-length or preferably the extracellular domain of the Neutrokine-αSV amino acid sequence shown in Figures 5A and 5B (SEQ ID NO:19 Polynucleotides encode the above Neutrokine-α SV mutants are also encompassed by the invention.

In addition, it will be recognized by one of ordinary skill in the art that mutations targeted to regions of a Neutrokine-α polypeptide of the invention which encompass the nineteen amino acid residue insertion which is not found in the Neutrokine-αSV polypeptide sequence (i.e., amino acid residues Val-142 through Lys-160 of the sequence presented in Figures 1A and 1B and in SEQ ID NO:2) may affect the observed functional activities (e.g., biological activity) of the Neutrokine-α polypeptide. More specifically, a partial, non-limiting and non-exclusive list of such residues of the Neutrokine-α polypeptide sequence which may be targeted for mutation includes the following amino acid residues of the Neutrokine-α polypeptide sequence as shown in SEQ ID NO:2: V-142; T-143; Q-144; D-145; C-146; L-147; Q-148; L-149; I-150; A-151; D-152; S-153; E-154; T-155; P-156; T-157; I-158; Q-159; and K-160.

Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

Thus, the invention also encompasses Neutrokine-α and/or Neutrokine-αSV derivatives and analogs that have one or more amino acid residues deleted, added, or substituted to generate Neutrokine-α and/or Neutrokine-αSV polypeptides that are better suited for expression, scale up, etc., in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognitions sequences in the Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention, and/or an amino acid deletion at the second

position of any one or more such recognition sequences will prevent glycosylation of the Neutrokine-α and/or Neutrokine-αSV at the modified tripeptide sequence (see, e.g., Miyajimo et al., EMBO J 5(6):1193-1197).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the Neutrokine-α and/or Neutrokine-αSV polypeptides can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

Percent Identity

The polypeptides of the present invention include the complete polypeptide encoded by the deposited cDNA (ATCC Deposit No. 97768) including the intracellular, transmembrane and extracellular domains of the polypeptide encoded by the deposited cDNA, the extracellular domain minus the intracellular and transmembrane domains of the protein, the complete polypeptide of Figures 1A and 1B (amino acid residues 1-285 of SEQ ID NO:2), the extracellular domain of Figures 1A and 1B (amino acid residues 73-285 of SEQ ID NO:2) minus the intracellular and transmembrane domains, as well as polypeptides which have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above.

The polypeptides of the present invention also include the complete polypeptide encoded by the deposited cDNA including the intracellular, transmembrane and extracellular domains of the polypeptide encoded by the deposited cDNA (ATCC Deposit No. 203518), the extracellular domain minus the intracellular and transmembrane domains of the protein, the complete polypeptide of Figures 5A and 5B (amino acid residues 1-266 of SEQ ID NO:19), the extracellular domain of Figures 5A and 5B (amino acid residues 73-266 of SEQ ID NO:19) minus the intracellular and transmembrane domains, as well as polypeptides which have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above.

Further polypeptides of the present invention include polypeptides at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA (ATCC Deposit No. 97768) or to the polypeptide of Figures 1A and 1B (SEQ ID NO:2), and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

Further polypeptides of the present invention include polypeptides at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA (ATCC Deposit No. 203518) or to the polypeptide of Figures 5A and 5B (SEQ ID NO:19), and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By "% similarity" for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2:482-489, 1981) to find the best segment of similarity between two sequences.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a Neutrokine-a and/or Neutrokine-aSV polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the Neutrokine-a and/or Neutrokine-aSV polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figures 1A and 1B (SEQ ID NO:2), the amino acid sequence encoded by the deposited cDNA clone HNEDU15 (ATCC Accession No. 97768), or fragments thereof, or, for instance, to the amino acid sequence shown in Figures 5A and 5B (SEQ ID NO:19), the amino acid sequence encoded by the deposited cDNA clone HDPMC52 (ATCC Accession No.

203518), or fragments thereof, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent

identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

A further embodiment of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of a Neutrokine-α or Neutrokine-αSV polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of a Neutrokine-α polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those skilled in the art. Additionally, as described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting Neutrokine-α and/or Neutrokine-αSV polypeptide expression as described below or as agonists and antagonists capable of enhancing or inhibiting Neutrokine-α and/or Neutrokine-αSV function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" Neutrokine-α and/or Neutrokine-αSV binding proteins which are also candidate

agonists and antagonists according to the present invention. The yeast two hybrid system is described in Fields and Song, Nature 340:245-246 (1989).

Transgenics and "knock-outs"

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be

selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of Neutrokine-α and/or Neutrokine-αSV polypeptides, studying conditions and/or disorders associated with aberrant Neutrokine-α and/or Neutrokine-αSV expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular

environment, does not allow the introduced cells to be recognized by the host immune system.

Antibodies

The present invention further relates to antibodies and T-cell antigen receptors (TCR) which specifically bind the polypeptides of the present invention. The antibodies of the present invention include IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. As used herein, the term "antibody" (Ab) is meant to include whole antibodies, including single-chain whole antibodies, and antigen-binding fragments thereof. Most preferably the antibodies are human antigen binding antibody fragments of the present invention include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, rabbit, goat, guinea pig, camel, horse, or chicken.

Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entire or partial of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are any combinations of variable region(s) and hinge region, CH1, CH2, and CH3 domains. The present invention further includes chimeric, humanized, and human monoclonal and polyclonal antibodies which specifically bind the polypeptides of the present invention. The present invention further includes antibodies which are anti-idiotypic to the antibodies of the present invention.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, A. et al. (1991) J. Immunol. 147:60-69; US Patents 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; Kostelny, S.A. et al. (1992) J. Immunol. 148:1547-1553.

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which are recognized or specifically bound by the antibody. The epitope(s) or polypeptide portion(s) may be specified as described herein, *e.g.*, by N-terminal

and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of the polypeptides of the present invention are included. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies which only bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity. Preferred binding affinities include those with a dissociation constant or Kd less than $5X10^{-6}$M, 10^{-6}M, $5X10^{-7}$M, 10^{-7}M, $5X10^{-8}$M, 10^{-8}M, $5X10^{-9}$M, 10^{-9}M, $5X10^{-10}$M, 10^{-10}M, $5X10^{-11}$M, 10^{-11}M, $5X10^{-12}$M, 10^{-12}M, $5X10^{-13}$M, 10^{-13}M, $5X10^{-14}$M, 10^{-14}M, $5X10^{-15}$M, and 10^{-15}M.

Antibodies of the present invention have uses that include, but are not limited to, methods known in the art to purify, detect, and target the polypeptides of the present invention including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. *See, e.g.*, Harlow et al., *ANTIBODIES: A LABORATORY MANUAL*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference in the entirety).

The antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. *See, e.g.*, WO 92/08495; WO 91/14438; WO 89/12624; US Patent 5,314,995; and EP 0 396 387.

The antibodies of the present invention may be prepared by any suitable method known in the art. For example, a polypeptide of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. Monoclonal antibodies can be prepared using a wide of techniques known in the art including the use of hybridoma and recombinant technology. *See, e.g.*, Harlow et al., ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: MONOCLONAL ANTIBODIES AND T-CELL HYBRIDOMAS 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties).

The antibodies of the present invention may be prepared by any of a variety of standard methods. For example, cells expressing the Neutrokine-α and/or Neutrokine-aSV polypeptide or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of Neutrokine-α and/or Neutrokine-aSV polypeptide is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or Neutrokine-α and/or Neutrokine-aSV polypeptide binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Köhler *et al., Nature 256*:495 (1975); Köhler *et al., Eur. J. Immunol. 6*:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., (1981) pp. 563-681). In general, such procedures involve immunizing an animal (preferably a mouse) with a Neutrokine-α and/or Neutrokine-aSV polypeptide antigen or, more preferably, with a Neutrokine-α and/or Neutrokine-aSV polypeptide-expressing cell. Suitable cells can be recognized by their capacity to bind anti-Neutrokine-α and/or anti-Neutrokine-aSV polypeptide antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC, Manassas,

Virginia. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the Neutrokine-α and/or Neutrokine-αSV antigen.

Alternatively, additional antibodies capable of binding to the Neutrokine-α and/or Neutrokine-αSV polypeptide antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, Neutrokine-α and/or Neutrokine-αSV polypeptide-specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the Neutrokine-α and/or Neutrokine-αSV polypeptide-specific antibody can be blocked by the Neutrokine-α and/or Neutrokine-αSV antigen. Such antibodies comprise anti-idiotypic antibodies to the Neutrokine-α and/or Neutrokine-αSV polypeptide-specific antibody and can be used to immunize an animal to induce formation of further Neutrokine-α and/or Neutrokine-αSV polypeptide-specific antibodies.

Fab and F(ab')2 fragments may be produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments).

Alternatively, antibodies of the present invention can be produced through the application of recombinant DNA technology or through synthetic chemistry using methods known in the art. For example, the antibodies of the present invention can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle which carries polynucleotide sequences encoding them. Phage with a desired binding property are selected from a repertoire or combinatorial antibody library (e.g. human or murine) by selecting directly with antigen, typically antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman U. et al. (1995) J. Immunol. Methods 182:41-50; Ames, R.S. et al. (1995) J. Immunol. Methods 184:177-

186; Kettleborough, C.A. et al. (1994) Eur. J. Immunol. 24:952-958; Persic, L. et al. (1997) Gene 187 9-18; Burton, D.R. et al. (1994) Advances in Immunology 57:191-280; PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patents 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743 (said references incorporated by reference in their entireties).

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax, R.L. et al. (1992) BioTechniques 12(6):864-869; and Sawai, H. et al. (1995) AJRI 34:26-34; and Better, M. et al. (1988) Science 240:1041-1043 (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al. (1991) Methods in Enzymology 203:46-88; Shu, L. et al. (1993) PNAS 90:7995-7999; and Skerra, A. et al. (1988) Science 240:1038-1040. For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. Methods for producing chimeric antibodies are known in the art. *See e.g.*, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies, S.D. et al. (1989) J. Immunol. Methods 125:191-202; and US Patent 5,807,715. Antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; US Patent 5,530,101; and 5,585,089), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E.A., (1991) Molecular Immunology 28(4/5):489-498; Studnicka G.M. et al. (1994) Protein Engineering 7(6):805-814; Roguska M.A. et al. (1994) PNAS 91:969-973), and chain shuffling (US Patent 5,565,332). Human antibodies can be made by a variety of methods known in the art including phage display methods described above. *See also*, US Patents 4,444,887, 4,716,111, 5,545,806, and 5,814,318; and WO 98/46645 (said references incorporated by reference in their entireties).

Further included in the present invention are antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently

conjugations) to a polypeptide of the present invention. The antibodies may be specific for antigens other than polypeptides of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in *in vitro* immunoassays and purification methods using methods known in the art. *See e.g.*, Harbor et al. *supra* and WO 93/21232; EP 0 439 095; Naramura, M. et al. (1994) Immunol. Lett. 39:91-99; US Patent 5,474,981; Gillies, S.O. et al. (1992) PNAS 89:1428-1432; Fell, H.P. et al. (1991) J. Immunol. 146:2446-2452 (said references incorporated by reference in their entireties).

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides or for use in immunoassays using methods known in the art. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. *See e.g.*, US Patents 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,112,946; EP 0 307 434, EP 0 367 166; WO 96/04388, WO 91/06570; Ashkenazi, A. et al. (1991) PNAS 88:10535-10539; Zheng, X.X. et al. (1995) J. Immunol. 154:5590-5600; and Vil, H. et al. (1992) PNAS 89:11337-11341 (said references incorporated by reference in their entireties).

The invention further relates to antibodies which act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Included are both receptor-specific antibodies and ligand-specific antibodies. Included are receptor-specific antibodies which do not prevent ligand binding

but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. Also included are receptor-specific antibodies which both prevent ligand binding and receptor activation. Likewise, included are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included are antibodies which activate the receptor. These antibodies may act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies may be specified as agonists or antagonists for biological activities comprising specific activities disclosed herein. The above antibody agonists can be made using methods known in the art. See e.g., WO 96/40281; US Patent 5,811,097; Deng, B. et al. (1998) Blood 92(6):1981-1988; Chen, Z. et al. (1998) Cancer Res. 58(16):3668-3678; Harrop, J.A. et al. (1998) J. Immunol. 161(4):1786-1794; Zhu, Z. et al. (1998) Cancer Res. 58(15):3209-3214; Yoon, D.Y. et al. (1998) J. Immunol. 160(7):3170-3179; Prat, M. et al. (1998) J. Cell. Sci. 111(Pt2):237-247; Pitard, V. et al. (1997) J. Immunol. Methods 205(2):177-190; Liautard, J. et al. (1997) Cytokinde 9(4):233-241; Carlson, N.G. et al. (1997) J. Biol. Chem. 272(17):11295-11301; Taryman, R.E. et al. (1995) Neuron 14(4):755-762; Muller, Y.A. et al. (1998) Structure 6(9):1153-1167; Bartunek, P. et al. (1996) Cytokine 8(1):14-20 (said references incorporated by reference in their entireties).

As discussed above, antibodies to the Neutrokine-α and/or Neutrokine-α SV polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" the Neutrokine-α, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to Neutrokine-α and/or Neutrokine-α SV and competitively inhibit the Neutrokine-α and/or Neutrokine-α SV multimerization and/or binding to ligand can be used to generate anti-idiotypes that "mimic" the Neutrokine-α TNF multimerization and/or binding domain and, as a consequence, bind to and neutralize Neutrokine-α or Neutrokine-α SV and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize Neutrokine-α ligand. For example, such anti-idiotypic antibodies can be used to bind

Neutrokine-α and/or Neutrokine-α SV, or to bind Neutrokine-α and/or Neutrokine-α SV receptors on the surface of cells of B cell lineage, and thereby block Neutrokine-α and/or Neutrokine-α SV mediated B cell activation, proliferation, and/or differentiation.

Immune System-Related Disorder Diagnosis

Neutrokine-α is expressed in kidney, lung, peripheral leukocyte, bone marrow, T cell lymphoma, B cell lymphoma, activated T cells, stomach cancer, smooth muscle, macrophages, and cord blood tissue, and particularly cells of monocytic lineage. Moreover, Neutrokine-αSV is expressed in primary dendritic cells. For a number of immune system-related disorders, substantially altered (increased or decreased) levels of Neutrokine-α and/or Neutrokine-αSV gene expression can be detected in immune system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" Neutrokine-α and/or Neutrokine-αSV gene expression level, that is, the Neutrokine-α and/or Neutrokine-αSV expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of an system disorder, which involves measuring the expression level of the gene encoding the Neutrokine-α and/or Neutrokine-αSV polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokine-α and/or Neutrokine-αSV gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

In particular, it is believed that certain tissues in mammals with cancer of cells or tissue of the immune system express significantly enhanced or reduced levels of the Neutrokine-α and/or Neutrokine-αSV polypeptide and mRNA encoding the Neutrokine-α and/or Neutrokine-αSV polypeptide when compared to a corresponding "standard" level. Further, it is believed that enhanced or depressed levels of the Neutrokine-α and/or Neutrokine-αSV polypeptide can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

Thus, the invention provides a diagnostic method useful during diagnosis of a immune system disorder, including cancers of this system, which involves measuring the expression level of the gene encoding the Neutrokine-α and/or Neutrokine-αSV polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokine-α and/or Neutrokine-αSV gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

Where a diagnosis of a disorder in the immune system, including diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed Neutrokine-α and/or Neutrokine-αSV gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "assaying the expression level of the gene encoding the Neutrokine-α and/or Neutrokine-αSV polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of the Neutrokine-α and/or Neutrokine-αSV polypeptide or the level of the mRNA encoding the Neutrokine-α and/or Neutrokine-αSV polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the Neutrokine-α and/or Neutrokine-αSV polypeptide level or mRNA level in a second biological sample). Preferably, the Neutrokine-α and/or Neutrokine-αSV polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard Neutrokine-α and/or Neutrokine-αSV polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once a standard Neutrokine-α and/or Neutrokine-αSV polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains Neutrokine-α and/or Neutrokine-αSV polypeptide or mRNA. As

indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free extracellular domains of the Neutrokine-α and/or Neutrokine-αSV polypeptide, immune system tissue, and other tissue sources found to express complete or free extracellular domain of the Neutrokine-α and/or Neutrokine-αSV or a Neutrokine-α and/or Neutrokine-αSV receptor. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The present invention is useful for diagnosis or treatment of various immune system-related disorders in mammals, preferably humans. Such disorders include but are not limited to tumors and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases, and graft versus host disease.

Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the Neutrokine-α and/or Neutrokine-αSV polypeptide are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

Assaying Neutrokine-α and/or Neutrokine-αSV polypeptide levels in a biological sample can occur using antibody-based techniques. For example, Neutrokine-α and/or Neutrokine-αSV polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105:*3087-3096 (1987)). Other antibody-based methods useful for detecting Neutrokine-α and/or Neutrokine-αSV polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying Neutrokine-α and/or Neutrokine-αSV polypeptide levels in a biological sample obtained from an individual, Neutrokine-α and/or Neutrokine-αSV polypeptide can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of Neutrokine-α and/or Neutrokine-αSV polypeptide include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where *in vivo* imaging is used to detect enhanced levels of Neutrokine-α and/or Neutrokine-αSV polypeptide for diagnosis in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science 229*:1202 (1985); Oi et al., *BioTechniques 4*:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne *et al., Nature 312*:643 (1984); Neuberger *et al., Nature 314*:268 (1985).

A Neutrokine-α and/or Neutrokine-αSV polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain Neutrokine-α protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Treatment of Immune System-Related Disorders

As noted above, Neutrokine-α and/or Neutrokine-αSV polynucleotides and polypeptides are useful for diagnosis of conditions involving abnormally high or low expression of Neutrokine-α and/or Neutrokine-αSV activities. Given the cells and tissues where Neutrokine-α and/or Neutrokine-αSV is expressed as well as the activities modulated by Neutrokine-α and/or Neutrokine-αSV, it is readily apparent that a substantially altered (increased or decreased) level of expression of Neutrokine-α and/or Neutrokine-αSV in an individual compared to the standard or "normal" level produces pathological conditions related to the bodily system(s) in which Neutrokine-α and/or Neutrokine-αSV is expressed and/or is active.

It will also be appreciated by one of ordinary skill that, since the Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention are members of the TNF family, the extracellular domains of the respective proteins may be released in soluble form from the cells which express Neutrokine-α and/or Neutrokine-αSV by proteolytic cleavage and therefore, when Neutrokine-α and/or Neutrokine-αSV polypeptide (particularly a soluble form of the respective extracellular domains) is added from an exogenous source to cells, tissues or the body of an individual, the polypeptide will exert its modulating activities on any of its target cells of that individual. Also, cells expressing this type II transmembrane protein may be added to cells, tissues or the body of an individual whereby the added cells will bind to cells expressing receptor for Neutrokine-α and/or Neutrokine-αSV whereby the cells expressing Neutrokine-α and/or Neutrokine-αSV can cause actions (e.g., cytotoxicity) on the receptor-bearing target cells.

Therefore, it will be appreciated that conditions caused by a decrease in the standard or normal level of Neutrokine-α and/or Neutrokine-αSV activity in an individual, particularly disorders of the immune system, can be treated by administration of Neutrokine-α and/or Neutrokine-αSV polypeptide (in the form of soluble extracellular domain or cells expressing the complete protein). Thus, the invention also provides a method of treatment of an individual in need of an increased level of Neutrokine-α and/or Neutrokine-αSV activity comprising

administering to such an individual a pharmaceutical composition comprising an amount of an isolated Neutrokine-α and/or Neutrokine-αSV polypeptide of the invention, effective to increase the Neutrokine-α and/or Neutrokine-αSV activity level in such an individual.

Since Neutrokine-α and/or Neutrokine-αSV belong to the TNF superfamily, the polypeptides should also modulate angiogenesis. In addition, since Neutrokine-α and/or Neutrokine-αSV inhibit immune cell functions, the polypeptides will have a wide range of anti-inflammatory activities. Neutrokine-α and/or Neutrokine-αSV may be employed as an anti-neovascularizing agent to treat solid tumors by stimulating the invasion and activation of host defense cells, e.g., cytotoxic T cells and macrophages and by inhibiting the angiogenesis of tumors. Those of skill in the art will recognize other non-cancer indications where blood vessel proliferation is not wanted. They may also be employed to enhance host defenses against resistant chronic and acute infections, for example, myobacterial infections via the attraction and activation of microbicidal leukocytes. Neutrokine-α and/or Neutrokine-αSV may also be employed to inhibit T-cell proliferation by the inhibition of IL-2 biosynthesis for the treatment of T-cell mediated auto-immune diseases and lymphocytic leukemias. Neutrokine-α and/or Neutrokine-αSV may also be employed to stimulate wound healing, both via the recruitment of debris clearing and connective tissue promoting inflammatory cells. In this same manner, Neutrokine-α and/or Neutrokine-αSV may also be employed to treat other fibrotic disorders, including liver cirrhosis, osteoarthritis and pulmonary fibrosis. Neutrokine-α and/or Neutrokine-αSV also increases the presence of eosinophils which have the distinctive function of killing the larvae of parasites that invade tissues, as in schistosomiasis, trichinosis and ascariasis. It may also be employed to regulate hematopoiesis, by regulating the activation and differentiation of various hematopoietic progenitor cells, for example, to release mature leukocytes from the bone marrow following chemotherapy, i.e., in stem cell mobilization. Neutrokine-α and/or Neutrokine-αSV may also be employed to treat sepsis.

Additional preferred embodiments of the invention include, but are not limited to, the use of Neutrokine-α polypeptides and functional agonists in the following applications:

A vaccine adjuvant that enhances immune responsiveness to specific antigen.

An adjuvant to enhance tumor-specific immune responses.

An adjuvant to enhance anti-viral immune responses.

As a stimulator of B cell responsiveness to pathogens.

As an agent that elevates the immune status of a individual prior to their receipt of immunosuppressive therapies.

As an agent to accelerate recovery of immunocompromised individuals;

As an agent to boost immunoresponsiveness among aged populations;

As an immune system enhancer following bone marrow transplant.

As a mediator of mucosal immune responses. The expression of Neutrokine-a by monocytes and the responsiveness of B cell to this factor suggests that it may be involved in exchange of signals between B cells and monocytes or their differentiated progeny. This activity is in many ways analogous to the CD40-CD154 signalling between B cells and T cells. Neutrokine-a may therefore be an important regulator of T cell independent immune responses to environmental pathogens. In particular, the unconventional B cell populations (CD5+) that are associated with mucosal sites and responsible for much of the innate immunity in humans may respond to Neutrokine-a thereby enhancing an individual's protective immune status.

As an agent to direct an individuals immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

As B cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic B cell populations.

As a means of detecting B-lineage cells by virtue of its specificity. This application may require labeling the protein with biotin or other agents to afford a means of detection.

As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodificiency;

As part of a B cell selection device the function of which is to isolate B cells from a heterogenous mixture of cell types. Neutrokine-a could be coupled to a solid support to which B cells would then specifically bind. Unbound cells would be washed out and the bound cells subsequently eluted. This technique would allow purging of tumor cells from, for example, bone marrow or peripheral blood prior to transplant.

As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

As an antigen for the generation of antibodies to inhibit or enhance Neutrokine-a mediated responses.

As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.

As a means of regulating secreted cytokines that are elicited by Neutrokine-a.

All of the above described applications as they may apply to veterinary medicine.

Antagonists of Neutrokine-a include binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the Neutrokine-a receptor(s). These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens. Although our current data speaks directly to the potential role of Neutrokine-a in B cell and monocyte related pathologies, it remains possible that other cell types may gain expression or responsiveness to Neutrokine-α. Thus, Neutrokine-α may, like CD40 and its ligand, be regulated by the status of the immune system and the microenvironment in which the cell is located.

A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythramatosus and MS.

An inhibitor of graft versus host disease or transplant rejection.

A therapy for B cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.

A therapy for chronic hypergammaglobulinemeia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, and related idiopathic monoclonalgammopathies.

A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

An immunosuppressive agent(s).

An inhibitor of signalling pathways involving ERK1, COX2 and Cyclin D2 which have been associated with Neutrokine-α induced B cell activation.

The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described above.

The antagonists may be employed for instance to inhibit Neutrokine-α and/or Neutrokine-αSV the chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of auto-immune diseases include multiple sclerosis, and insulin-dependent diabetes. The antagonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by preventing eosinophil production and migration. Endotoxic shock may also be treated by the antagonists by preventing the migration of macrophages and their production of the Neutrokine-α and/or Neutrokine-αSV polypeptides of the present invention. The antagonists may also be employed for treating atherosclerosis, by preventing monocyte infiltration in the artery wall. The antagonists may also be employed to treat histamine-mediated allergic reactions and immunological disorders including late phase allergic reactions, chronic urticaria, and atopic dermatitis by inhibiting chemokine-induced mast cell and basophil degranulation and release of histamine. IgE-mediated allergic reactions such as allergic asthma, rhinitis, and eczema may also be treated. The antagonists may also be employed to treat chronic and acute inflammation by preventing the attraction of monocytes to a wound area. They may also be employed to regulate normal pulmonary macrophage populations, since chronic and acute inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung. Antagonists may also be employed to treat rheumatoid arthritis by preventing the attraction of monocytes into synovial fluid in the joints of patients.

Monocyte influx and activation plays a significant role in the pathogenesis of both degenerative and inflammatory arthropathies. The antagonists may be employed to interfere with the deleterious cascades attributed primarily to IL-1 and TNF, which prevents the biosynthesis of other inflammatory cytokines. In this way, the antagonists may be employed to prevent inflammation. The antagonists may also be employed to inhibit prostaglandin-independent fever induced by Neutrokine-α and/or Neutrokine-αSV. The antagonists may also be employed to treat cases of bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome. The antagonists may also be employed to treat asthma and allergy by preventing eosinophil accumulation in the lung. The antagonists may also be employed to treat subepithelial basement membrane fibrosis which is a prominent feature of the asthmatic lung.

Antibodies against Neutrokine-α and/or Neutrokine-αSV may be employed to bind to and inhibit Neutrokine-α and/or Neutrokine-αSV activity to treat ARDS, by preventing infiltration of neutrophils into the lung after injury. The antagonists and antagonists of the instant may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described hereinafter.

Formulations

The Neutrokine-α and/or Neutrokine-αSV polypeptide composition (preferably containing a polypeptide which is a soluble form of the Neutrokine-α and/or Neutrokine-αSV extracellular domains) will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with Neutrokine-α and/or Neutrokine-αSV polypeptide alone), the site of delivery of the Neutrokine-α Neutrokine-α and/or Neutrokine-αSV polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of Neutrokine-α and/or Neutrokine-αSV polypeptide for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of Neutrokine-α and/or Neutrokine-αSV polypeptide administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given

continuously, the Neutrokine-α and/or Neutrokine-αSV polypeptide is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. In one embodiment, "pharmaceutically acceptable carrier" means a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In a specific embodiment, "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly humans. Nonlimiting examples of suitable pharmaceutical carriers according to this embodiment are provided in "Remington's Pharmaceutical Sciences" by E.W. Martin, and include sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can be employed as liquid carriers, particularly for injectable solutions.

The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The Neutrokine-α and/or Neutrokine-αSV polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or

poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release Neutrokine-α and/or Neutrokine-αSV polypeptide compositions also include liposomally entrapped Neutrokine-α and/or Neutrokine-αSV polypeptide. Liposomes containing Neutrokine-α and/or Neutrokine-αSV polypeptide are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Neutrokine-α and/or Neutrokine-αSV polypeptide therapy.

For parenteral administration, in one embodiment, the Neutrokine-α and/or Neutrokine-αSV polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the Neutrokine-α and/or Neutrokine-αSV polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid,

aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Neutrokine-α and/or Neutrokine-αSV polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of Neutrokine-α and/or Neutrokine-αSV polypeptide salts.

Neutrokine-α and/or Neutrokine-αSV polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic Neutrokine-α and/or Neutrokine-αSV polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Neutrokine-α and/or Neutrokine-αSV polypeptide ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Neutrokine-α and/or Neutrokine-αSV polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Neutrokine-α and/or Neutrokine-αSV polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

Agonists and Antagonists - Assays and Molecules

The invention also provides a method of screening compounds to identify those which enhance or block the action of Neutrokine-α and/or Neutrokine-αSV polypeptide on cells, such as its interaction with Neutrokine-α and/or Neutrokine-αSV binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of Neutrokine-α and/or Neutrokine-αSV or which functions in a manner similar to Neutrokine-α and/or Neutrokine-αSV while antagonists decrease or eliminate such functions.

In another embodiment, the invention provides a method for identifying a receptor protein or other ligand-binding protein which binds specifically to a Neutrokine-α and/or Neutrokine-αSV polypeptide. For example, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds Neutrokine-α and/or Neutrokine-αSV. The preparation is incubated with labeled Neutrokine-α and/or Neutrokine-αSV and complexes of Neutrokine-α and/or Neutrokine-αSV bound to the receptor or other binding protein are isolated and characterized according to routine methods known in the art. Alternatively, the Neutrokine-α and/or Neutrokine-αSV polypeptide may be bound to a solid support so that binding molecules solubilized from cells are bound to the column and then eluted and characterized according to routine methods.

In the assay of the invention for agonists or antagonists, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds Neutrokine-α and/or Neutrokine-αSV such as a molecule of a signaling or regulatory pathway modulated by Neutrokine-α and/or Neutrokine-αSV. The preparation is incubated with labeled Neutrokine-α and/or Neutrokine-αSV in the absence or the presence of a candidate molecule which may be a Neutrokine-α and/or Neutrokine-αSV agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of Neutrokine-α on binding the Neutrokine-α and/or Neutrokine-αSV binding molecule, are most likely to be good antagonists. Molecules that bind well and

elicit effects that are the same as or closely related to Neutrokine-α and/or Neutrokine-αSV are agonists.

Neutrokine-α and/or Neutrokine-αSV -like effects of potential agonists and antagonists may by measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of Neutrokine-α and/or Neutrokine-αSV or molecules that elicit the same effects as Neutrokine-α and/or Neutrokine-αSV. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

Another example of an assay for Neutrokine-α and/or Neutrokine-αSV antagonists is a competitive assay that combines Neutrokine-α and/or Neutrokine-αSV and a potential antagonist with membrane-bound receptor molecules or recombinant Neutrokine-α and/or Neutrokine-αSV receptor molecules under appropriate conditions for a competitive inhibition assay. Neutrokine-α and/or Neutrokine-αSV can be labeled, such as by radioactivity, such that the number of Neutrokine-α and/or Neutrokine-αSV molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing Neutrokine-α and/or Neutrokine-αSV induced activities, thereby preventing the action of Neutrokine-α and/or Neutrokine-αSV by excluding Neutrokine-α and/or Neutrokine-αSV from binding.

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are

discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the extracellular domain of the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of Neutrokine-α and/or Neutrokine-αSV. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into Neutrokine-α and/or Neutrokine-αSV polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of Neutrokine-α and/or Neutrokine-αSV.

In one embodiment, the Neutrokine-α and/or Neutrokine-αSV antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the Neutrokine-α and/or Neutrokine-αSV antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others know in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding Neutrokine-α and/or Neutrokine-αSV, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a Neutrokine-α

and/or Neutrokine-αSV gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded Neutrokine-α and/or Neutrokine-αSV antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatches with a Neutrokine-α and/or Neutrokine-αSV RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of Neutrokine-α and Neutrokine-αSV shown in Figures 1A-B and 5A-B, respectively, could be used in an antisense approach to inhibit translation of endogenous Neutrokine-α and/or Neutrokine-αSV mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of Neutrokine-α and/or Neutrokine-αSV mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or

agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res. 15:6625-6641 (1987)). The oligonucleotide is a 2¢-0-

methylribonucleotide (Inoue et al., Nucl. Acids Res. 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1997)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res. 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the Neutrokine-α and/or Neutrokine-αSV coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy Neutrokine-α and/or Neutrokine-αSV mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of Neutrokine-α and Neutrokine-αSV (Figures 1A-B and 5A-B, respectively). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the Neutrokine-α and/or Neutrokine-αSV mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express Neutrokine-α and/or Neutrokine-αSV *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to

destroy endogenous Neutrokine-α and/or Neutrokine-αSV messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous gene expression can also be reduced by inactivating or "knocking out" theNeutrokine-α and/or Neutrokine-αSV gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

In other embodiments, antagonists according to the present invention include soluble forms of Neutrokine-α and/or Neutrokine-αSV (e.g., fragments of Neutrokine-α shown in Figures 1A-B that include the ligand binding domain, TNF conserved domain, and/or extracellular domain of Neutrokine-α and/or Neutrokine-αSV and fragments of Neutrokine-αSV shown in Figures 5A-B that include the ligand binding domain, TNF conserved domain, and/or extracellular domain of Neutrokine-α and/or Neutrokine-αSV). Such soluble forms of the Neutrokine-α and/or Neutrokine-αSV, which may be naturally occurring or synthetic, antagonize Neutrokine-α and/or Neutrokine-αSV mediated signaling by competing with native Neutrokine-α and/or Neutrokine-αSV for binding to Neutrokine-α and/or Neutrokine-αSV receptors (e.g., DR5 (*See*, International Publication No. WO 98/41629), TR10 (*See*, International Publication No. WO 98/54202), 312C2 (*See*, International

Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (*See*, U.S. Application Serial No. 09/176,200)), and/or by forming a multimer that may or may not be capable of binding the receptor, but which is incapable of inducing signal transduction. Preferably, these antagonists inhibit Neutrokine-α and/or Neutrokine-αSV mediated stimulation of lymphocyte (e.g., B-cell) proliferation, differentiation, and/or activation. Antagonists of the present invention also include antibodies specific for TNF-family ligands and Neutrokine-α–Fc and/or Neutrokine-αSV-Fc fusion proteins.

By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing and/or blocking the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, TNF-α, lymphotoxin-α (LT-α, also known as TNF-b), LT-b (found in complex heterotrimer LT-a2-b), FasL, CD40L, CD27L, CD30L, 4-IBBL, OX40L and nerve growth factor (NGF). In preferred embodiments, the Neutrokine-α and/or Neutrokine-αSV TNF-family ligands of the invention are DR5 (*See*, International Publication No. WO 98/41629), TR10 (*See*, International Publication No. WO 98/54202), 312C2 (*See*, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (*See*, U.S. Application Serial No. 09/176,200).

Antagonists of the present invention also include antibodies specific for TNF-family receptors or the Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention. Antibodies according to the present invention may be prepared by any of a variety of standard methods using Neutrokine-a and/or Neutrokine-αSV immunogens of the present invention. As indicated, such Neutrokine-α and/or Neutrokine-αSV immunogens include the complete Neutrokine-α and Neutrokine-αSV polypeptides depicted in Figures 1A-B (SEQ ID NO:2) and Figures 5A-B (SEQ ID NO:19), respectively, (which may or may not include the leader sequence) and Neutrokine-α and/or Neutrokine-αSV polypeptide fragments comprising, for example, the ligand binding domain, TNF-conserved domain, extracellular domain, transmembrane domain, and/or intracellular domain, or any combination thereof.

Polyclonal and monoclonal antibody agonists or antagonists according to the present invention can be raised according to the methods disclosed in Tartaglia and Goeddel, *J. Biol. Chem.* 267(7):4304-4307(1992)); Tartaglia et al., *Cell* 73:213-216 (1993)), and PCT Application WO 94/09137 and are preferably specific to (i.e., bind uniquely to polypeptides of the invention having the amino acid sequence of SEQ ID NO:2. The term "antibody" (Ab) or

"monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and F(ab') fragments) which are capable of binding an antigen. Fab, Fab' and F(ab') fragments lack the Fc fragment intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.*, 24:316-325 (1983)).

In a preferred method, antibodies according to the present invention are mAbs. Such mAbs can be prepared using hybridoma technology (Kohler and Millstein, *Nature* 256:495-497 (1975) and U.S. Patent No. 4,376,110; Harlow et al., *Antibodies*: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY, 1980; Campbell, "Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon et al., eds.), Elsevier, Amsterdam (1984)).

Proteins and other compounds which bind the Neutrokine-α and/or Neutrokine-αSV domains are also candidate agonists and antagonists according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989)). A modified version of the yeast two- hybrid system has been described by Roger Brent and his colleagues (Gyuris, *Cell* 75:791-803 (1993); Zervos et al., *Cell* 72:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to the ligand binding domain, extracellular, intracellular, transmembrane, and death domain of the Neutrokine-α and/or Neutrokine-αSV. Such compounds are good candidate agonists and antagonists of the present invention.

For example, using the two-hybrid assay described above, the extracellular or intracellular domain of the Neutrokine-α and/or Neutrokine-αSV receptor, or a portion thereof, may be used to identify cellular proteins which interact with Neutrokine-α and/or Neutrokine-αSV the receptor *in vivo*. Such an assay may also be used to identify ligands with potential agonistic or antagonistic activity of Neutrokine-α and/or Neutrokine-αSV receptor function. This screening assay has previously been used to identify protein which interact with the cytoplasmic domain of the murine TNF-RII and led to the identification of two receptor associated proteins. Rothe et al., *Cell* 78:681 (1994). Such proteins and amino acid sequences which bind to the cytoplasmic domain of the Neutrokine-α and/or Neutrokine-αSV receptors are good candidate agonist and antagonist of the present invention.

Other screening techniques include the use of cells which express the polypeptide of the present invention (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science*, 246:181-296 (1989). In another example, potential agonists or antagonists may be contacted with a cell which expresses the polypeptide of the present invention and a second messenger response, e.g., signal transduction may be measured to determine whether the potential antagonist or agonist is effective.

Agonist according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonists include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and -amyloid peptide. (*Science* 267:1457-1458 (1995)).

Preferred agonists are fragments of Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention which stimulate lymphocyte (e.g, B cell) proliferation, differentiation and/or activation. Further preferred agonists include polyclonal and monoclonal antibodies raised against the Neutrokine-α and/or Neutrokine-αSV polypeptides of the invention, or a fragment thereof. Such agonist antibodies raised against a TNF-family receptor are disclosed in Tartaglia et al., *Proc. Natl. Acad. Sci. USA* 88:9292-9296 (1991); and Tartaglia et al., *J. Biol. Chem.* 267:4304- 4307(1992). See, also, PCT Application WO 94/09137.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation,

liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

In yet another embodiment of the invention, the activity of Neutrokine-α and/or Neutrokine-αSV polypeptide can be reduced using a "dominant negative." To this end, constructs which encode defective Neutrokine-α and/or Neutrokine-αSV polypeptide, such as, for example, mutants lacking all or a portion of the TNF-conserved domain, can be used in gene therapy approaches to diminish the activity of Neutrokine-α and/or Neutrokine-αSV on appropriate target cells. For example, nucleotide sequences that direct host cell expression of Neutrokine-α and/or Neutrokine-αSV polypeptide in which all or a portion of the TNF-conserved domain is altered or missing can be introduced into monocytic cells or other cells or tissues (either by in vivo or ex vivo gene therapy methods described herein or otherwise known in the art). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject's endogenous Neutrokine-α and/or Neutrokine-αSV gene in monocytes. The engineered cells will express non-functional Neutrokine-α and/or Neutrokine-αSV polypeptides (i.e., a ligand (e.g., multimer) that may be capable of binding, but which is incapable of inducing signal transduction).

Chromosome Assays

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA and/or polynucleotides herein disclosed is used to clone genomic DNA of a Neutrokine-α and/or Neutrokine-αSV gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is

observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Utilizing the techniques described above, the chromosomal location of Neutrokine-a and Neutrokine-aSV was determined with high confidence using a combination of somatic cell hybrids and radiation hybrids to chromosome position 13q34.

Examples

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting. Many of the following examples are set forth referring specifically to Neutrokine-a polynucleotides and polypeptides of the invention. Each example may also be practised to generate and/or examine Neutrokine-aSV polynucleotides and/or polypeptides of the invention. One of ordinary skill in the art would easily be able to direct the following examples to Neutrokine-aSV.

Example 1a: Expression and Purification of "His-tagged" Neutrokine-α in E. coli

The bacterial expression vector pQE9 (pD10) is used for bacterial expression in this example. (QIAGEN, Inc., *supra*). pQE9 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide.

The DNA sequence encoding the desired portion of the Neurokine-a protein comprising the extracellular domain sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the

amino terminal sequences of the desired portion of the Neurokine-a protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE9 vector are added to the 5' and 3' primer sequences, respectively.

For cloning the extracellular domain of the protein, the 5' primer has the sequence 5' GTG GGA TCC AGC CTC CGG GCA GAG CTG-3' (SEQ ID NO:10) containing the underlined *Bam* HI restriction site followed by 18 nucleotides of the amino terminal coding sequence of the extracellular domain of the Neurokine-a sequence in Figures 1A and 1B. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete Neutrokine a protein shorter or longer than the extracellular domain of the form. The 3' primer has the sequence 5'-GTG AAG CTT TTA TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:11) containing the underlined *Hind* III restriction site followed by two stop codons and 18 nucleotides complementary to the 3' end of the coding sequence of the Neurokine-a DNA sequence in Figures 1A and 1B.

The amplified Neurokine-a DNA fragment and the vector pQE9 are digested with *Bam* HI and *Hind* III and the digested DNAs are then ligated together. Insertion of the Neurokine-a DNA into the restricted pQE9 vector places the Neurokine-a protein coding region downstream from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine codons.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing Neurokine-a protein, is available commercially from QIAGEN, Inc., *supra*. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing. Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both

ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-β-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4° C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the Neurokine-a is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the Neurokine-a is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins can be eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4° C or frozen at -80° C.

Example 1b: Expression and Purification of Neutrokine-a in E. coli

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding

site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such as way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

The DNA sequence encoding the desired portion of the Neurokine-a protein comprising the extracellular domain sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the Neurokine-a protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning the extracellular domain of the protein, the 5' primer has the sequence 5' GTG TCA TGA GCC TCC GGG CAG AGC TG 3' (SEQ ID NO:12) containing the underlined *Bsp* HI restriction site followed by 17 nucleotides of the amino terminal coding sequence of the extracellular domain of the Neurokine-a sequence in Figures 1A and 1B. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein shorter or longer than the extracellular domain of the form. The 3' primer has the sequence 5'-GTG AAG CTT TTA TTA CAG CAG TTT CAA TGC ACC 3' (SEQ ID NO:13) containing the underlined *Hind* III restriction site followed by two stop codons and 18 nucleotides complementary to the 3' end of the coding sequence in the Neurokine-a DNA sequence in Figures 1A and 1B.

The amplified Neurokine-a DNA fragments and the vector pQE60 are digested with *Bsp* HI and *Hind* III and the digested DNAs are then ligated together. Insertion of the Neurokine-a DNA into the restricted pQE60 vector places the Neurokine-a protein coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing Neurokine-a protein, is available commercially from QIAGEN, Inc., *supra*. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

One of ordinary skill in the art recognizes that any of a number of bacterial expression vectors may be useful in place of pQE9 and pQE60 in the expression protocols presented in this example. For example, the novel pHE4 series of bacterial expression vectors, in particular, the pHE4-5 vector may be used for bacterial expression in this example (ATCC Accession No. 209311; and variations thereof). The plasmid DNA designated pHE4-5/MPIFD23 in ATCC Deposit No. 209311 is vector plasmid DNA which contains an insert which encodes another ORF. The construct was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on September 30, 1997. Using the *Nde* I and *Asp* 718 restriction sites flanking the irrelevant MPIF ORF insert, one of ordinary skill in the art could easily use current molecular biological techniques to replace the irrelevant ORF in the pHE4-5 vector with the Neutrokine-a ORF of the present invention.

The pHE4-5 bacterial expression vector includes a neomycin phosphotransferase gene for selection, an *E. coli* origin of replication, a T5 phage promoter sequence, two *lac* operator sequences, a Shine-Delgarno sequence, and the lactose operon repressor gene (*lac*Iq). These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide. The promoter and operator sequences of the pHE4-5 vector were made synthetically. Synthetic production of nucleic acid sequences is well known in the art (CLONETECH 95/96 Catalog, pages 215-216, CLONETECH, 1020 East Meadow Circle, Palo Alto, CA 94303).

Clones containing the desired Neutrokine-a constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4° C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the Neutrokine a is dialyzed against 50 mM Na-acetate buffer pH 6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH 7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure Neurokine-a protein. The purified protein is stored at 4° C or frozen at -80° C.

In certain embodiments, it is preferred to generate expression constructs as detailed in this Example to mutate one or more of the three cysteine residues in the Neutrokine-a polypeptide sequence. The cysteine residues in the Neutrokine-a polypeptide sequence are located at positions 147, 232, and 245 as shown in SEQ ID NO:2 and at positions 213 and 226 of the Neutrokine-a polypeptide sequence as shown in SEQ ID NO:19 (there is no cysteine in the Neutrokine-aSV polypeptide sequence which corresponds to Cys-147 in the Neutrokine-a polypeptide sequence because amino acid residues 143-160 of the Neutrokine-a polypeptide sequence are not present in the Neutrokine-aSV polypeptide sequence).

Example 2: Cloning and Expression of Neutrokine-a Protein in a Baculovirus Expression System

In this illustrative example, the plasmid shuttle vector pA2GP is used to insert the cloned DNA encoding the extracellular domain of the protein, lacking its naturally associated intracellular and transmembrane sequences, into a

baculovirus to express the extracellular domain of the Neurokine-a protein, using a baculovirus leader and standard methods as described in Summers et al., *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 protein and convenient restriction sites such as *Bam* HI, *Xba* I and *Asp* 718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology 170*:31-39 (1989).

The cDNA sequence encoding an N-terminally deleted form of the extracellular domain of the Neurokine-a protein in the deposited clone, lacking the AUG initiation codon, the naturally associated intracellular and transmembrane domain sequences, and amino acids Gln-73 through Leu-79 shown in Figures 1A and 1B (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'-GTG GGA TCC CCG GGC AGA GCT GCA GGG C-3' (SEQ ID NO:14) containing the underlined *Bam* HI restriction enzyme site followed by 18 nucleotides of the sequence of the extracellular domain of the Neurokine-a protein shown in Figures 1A and 1B, beginning with the indicated N-terminus of the extracellular domain of the protein. The 3' primer has the sequence 5'-GTG GGA TCC TTA TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:15) containing the underlined *Bam* HI restriction site followed by two stop codons and 18 nucleotides complementary to the 3' coding sequence in Figures 1A and 1B.

In certain other embodiments, constructs designed to express the entire predicted extracellular domain of the Neutrokine-a (i.e., amino acid residues Gln-73 through Leu-285) are preferred. One of skill in the art would be able to use the polynucleotide and polypeptide sequences provided as SEQ ID NO:1 and SEQ ID NO:2, respectively, to design polynucleotide primers to generate such a clone.

In a further preferred embodiment, a pA2GP expression construct encodes amino acid residues Leu-112 through Leu-285 of the Neutrokine-a polypeptide sequence shown as SEQ ID NO:2.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with *Bam* HI and again is purified on a 1% agarose gel. This fragment is designated herein F1.

The plasmid is digested with the restriction enzymes *Bam* HI and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Statagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human Neurokine-a gene by digesting DNA from individual colonies using *Bam* HI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pA2GP-Neutrokine-a.

Five µg of the plasmid pA2GP-Neutrokine-a is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA 84*: 7413-7417 (1987). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid pA2GP Neurokine-a are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then

incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C. The recombinant virus is called V-Neurokine-a.

To verify the expression of the Neurokine-a gene Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-Neurokine-a at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 µCi of ^{35}S-methionine and 5 µCi ^{35}S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the extracellular domain of the protein and thus the cleavage point and length of the secretory signal peptide.

In a specific experimental example, recombinant Neurokine-a was purified from baculovirus infected Sf9 cell supernatants as follows. The insect cells were grown in EXCEL401 medium (JRH Scientific) with 1% (v/v) fetal bovine serum. At 92 hours post-infection, the harvested supernatant was clarified by centrifugation at 18,000 x *g* followed by 0.45 m depth filtration. A

de-lipid filtration step might be also used to remove the lipid contaminants and in turn to improve initial capturing of the Neutrokine-a protein.

The supernatant was loaded onto a set of poros HS-50/HQ-50 in tandem mode. As alternatives, Toyopearl QAE, Toyopearl Super Q (Tosohass), Q-Sepharose (Pharmacia) and equivalent resins might be used. This step is used as a negative purification step to remove strong anion binding contaminants. The HS/HQ flow through material was adjusted to pH 7.5 with 1 M Tris-HCl pH 8, diluted with equal volume of 50 mM Tris-HCl pH 8, and loaded onto a poros PI-20 or PI-50 column. The PI column was washed first with 4 column volumes of 75 mM sodium chloride in 50 mM Tris-HCl at pH 7.5, then eluted using 3 to 5 column volumes of a stepwise gradient of 300 mM, 750 mM, 1500 mM sodium chloride in 50 mM Tris-HCl pH 7.5. Neutrokine-a protein appears as a 17 KD band on reduced SDS-PAGE and is present in the 0.75 M to 1.5M Sodium chloride fractions.

The PI fraction was further purified through a Sephacryl S100 HR (Pharmacia) size exclusion column equilibrated with 0.15 M sodium chloride, 50 mM sodium acetate at pH 6. The S200 fractions were mixed with sodium chloride to a final concentration of 3 M and loaded onto a Toyopearl Hexyl 650C (Tosohass) column. The Hexyl column was eluted with a linear gradient from 3 M to 0.05 M sodium chloride in 50 mM Sodium acetate pH 6 in 5 to 15 column volumes. The sodium chloride gradient can also be replaced by ammonium sulfate gradient of 1M to 0 M in 50 mM sodium acetate pH 6 in the Hexyl chromatographic step. Fractions containing purified Neutrokine-a as analyzed through SDS-PAGE were combined and dialyzed against a buffer containing 150 mM Sodium chloride, 50 mM Sodium acetate, pH 6.

The final purified Neutrokine-a protein expressed in a baculovirus system as explained herein has an N-terminus sequence which begins with amino acid residue Ala-134 of SEQ ID NO:2. RP-HPLC analysis shows a single peak of greater than 95% purity. Endotoxin level was below the detection limit in LAL assay.

Example 3: Cloning and Expression of Neutrokine-a in Mammalian Cells

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers,

Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells, Chinese hamster ovary (CHO) cells, and HEK 293 cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J. 227*:277-279 (1991); Bebbington et al., *Bio/Technology 10*:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites *Bam* HI, *Xba* I and *Asp* 718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

The expression plasmid, pNeurokine-a-HA, is made by cloning a portion of the deposited cDNA encoding the extracellular domain of the Neurokine-a protein into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.). To produce a soluble, secreted form of the polypeptide, the extracellular domain is fused to the secretory leader sequence of the human IL-6 gene.

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson et al., *Cell 37*: 767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the extracellular domain of the Neurokine-a polypeptide is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The Neurokine-a cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of Neurokine-a *in E. coli*. Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined *Bam* HI site, a Kozak sequence, an AUG start codon, a sequence encoding the secretory leader peptide from the human IL-6 gene, and 18 nucleotides of the 5' coding region of the extracellular domain of Neurokine-a protein, has the following sequence: 5'-GCG GGA TCC GCC ACC ATG AAC TCC TTC TCC ACA AGC GCC TTC GGT CCA GTT GCC TTC TCC CTG GGG CTG CTC CTG GTG TTG CCT GCT GCC TTC CCT GCC CCA GTT GTG AGA CAA GGG GAC CTG GCC AGC-3' (SEQ ID NO:16). The 3' primer, containing the underlined *Bam*

HI restriction site and 18 of nucleotides complementary to the 3' coding sequence immediately before the stop codon, has the following sequence: 5'-GTG GGA TCC TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:17).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with *Bam* HI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the fragment encoding the Neutrokine-a extracellular domain.

For expression of recombinant Neurokine-a, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of Neurokine-a by the vector.

Expression of the Neurokine-a-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow et al., *Antibodies: A Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of Neurokine-a protein. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). To produce a soluble, secreted form of the Neurokine-a polypeptide,

the portion of the deposited cDNA encoding the extracellular domain is fused to the secretory leader sequence of the human IL-6 gene. The vector plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J. Biol. Chem.* *253*:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta*, *1097*:107-143, Page, M. J. and Sydenham, M. A. 1991, *Biotechnology* *9*:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, et al., *Molecular and Cellular Biology*, March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart et al., *Cell* *41*:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, Xba I, and Asp718. Behind these cloning sites the plasmid contains the 3′ intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human β-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the Neurokine-a in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA* *89*: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or

hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes *Bam* HI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the extracellular domain of the Neutrokine-α protein is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer, containing the underlined *Bam* HI site, a Kozak sequence, an AUG start codon, a sequence encoding the secretory leader peptide from the human IL-6 gene, and 18 nucleotides of the 5' coding region of the extracellular domain of Neurokine-α protein, has the following sequence: 5'-GCG GGA TCC GCC ACC ATG AAC TCC TTC TCC ACA AGC GCC TTC GGT CCA GTT GCC TTC TCC CTG GGG CTG CTC CTG GTG TTG CCT GCT GCC TTC CCT GCC CCA GTT GTG AGA CAA GGG GAC CTG GCC AGC-3' (SEQ ID NO:16). The 3' primer, containing the underlined *Bam* HI and 18 of nucleotides complementary to the 3' coding sequence immediately before the stop codon, has the following sequence: 5'-GTG GGA TCC TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:17).

The amplified fragment is digested with the endonuclease Bam HI and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400

nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 μM, 20 μM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200 μM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

At least six Neutrokine-a expression constructs have been generated by the inventors herein to facilitate the production of Neutrokine-a and/or Neutrokine-aSV polypeptides of several sizes and in several systems. The expression constructs are as follows: (1) pNa.A71-L285 (expresses amino acid residues Ala-71 through Leu-285), (2) pNa.A81-L285 (expresses amino acid residues Ala-81 through Leu-285), (3) pNa.L112-L285 (expresses amino acid residues Leu-112 through Leu-285), (4) pNa.A134-L285 (expresses amino acid residues Ala-134 through Leu-285), (5) pNa.L147-L285 (expresses amino acid residues Leu-147 through Leu-285), and (6) pNa.G161-L285 (expresses amino acid residues Gly-161 through Leu-285).

In preferred embodiments, the expression constructs are used to express various Neutrokine-a muteins from bacterial, baculoviral, and mammalian systems.

In certain additional preferred embodiments, the constructs express a Neutrokine-a polypeptide fragment fused at the N- and/or C-terminus to a heterologous polypeptide, e.g., the signal peptide from human IL-6, the signal peptide from CK-b8 (amino acids -21 to -1 of the CK-b8 sequence disclosed in published PCT application PCT/US95/09058), or the human IgG Fc region. Other sequences could be used which are known to those of skill in the art.

Example 4: Tissue distribution of Neutrokine-a mRNA expression

Northern blot analysis is carried out to examine Neutrokine-a gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the Neutrokine-a protein (SEQ ID NO:1) is labeled with ^{32}P using the *rediprime*™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for Neutrokine-a and/or Neutrokine-a mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70° C overnight, and films developed according to standard procedures.

To determine the pattern of Neutrokine-α and/or Neutrokine-β expression a panel of multiple tissue Northern blots were probed. This revealed predominant expression of single 2.6 kb mRNA in peripheral blood leukocytes, spleen, lymph node and bone marrow, and detectable expression in placenta, heart, lung, fetal liver, thymus and pancreas. Analysis of a panel of cell lines demonstrated high expression of Neutrokine-α and/or Neutrokine-α in HL60 cells, detectable expression in K562, but no expression in Raji, HeLa, or MOLT-4 cells. Overall it appears that Neutrokine-α and/or Neutrokine-α mRNA expression is enriched in the immune system.

Example 5: Gene Therapy Using Endogenous Neutrokine-α Gene

Another method of gene therapy according to the present invention involves operably associating the endogenous Neutrokine-α sequence with a promoter via homologous recombination as described, for example, in U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired. Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous Neutrokine-α, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of Neutrokine-α so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably,the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous Neutrokine-a sequence. This results in the expression of Neutrokine-a in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2 HPO4, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X106 cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the Neutrokine-a locus, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two Neutrokine-a non-coding sequences are amplified via PCR: one Neutrokine-a non-coding sequence (Neutrokine-a fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other Neutrokine-a non-coding sequence (Neutrokine-a fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter

and Neutrokine-a fragments are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; Neutrokine-a fragment 1 - XbaI; Neutrokine-a fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio- Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5.X106 cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37°C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 6: Neutrokine-α, a Novel human Tumor Necrosis Factor Homologue that Induces B Cell Proliferation and Differentiation

Background

Generation of a functional humoral immune resposes requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL5, IL6, IL-7, IL10, IL-13, IL14 and IL15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell

populations. One of the best studied classes of B cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their repective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Recently, Human Genome Sciences Inc. has identified a novel member of the TNF-lignad family, Neutrokine-α, which is a potent and specific inducer of B lymphocyte activation, proliferation and differentiation. Neutrokine-α is produced by monocytic cell types, and has its effects on B cells through a putative receptor expressed on B cells. Engagement of the receptor in the presence of a co-stimulatory signal delivered through membrane bound Ig receptors results in the proliferation and differntiation of normal human tonsillar B cells.

Results

As part of an ongoing genomics-based gene discovery program, the Human Genome Sciences, Inc. EST database was searched for sequences encoding characteristic TNF-like domains. Recently, a 285 amino acid protein was identified in a human neutrophil-derived cDNA library that shared significant homology to APRIL (28%), LTα (20%), and TNFα (10%), (Figure 7A). Like other members of the TNF-ligand family, it is a type II transmembrane protein containing a predicted N-terminal cytosolic domain of 46 residues, a transmembrane region of 22 residues, and a 212 residue extracellular domain. Expression of this cDNA in mammalian cells (both HEK 293 and Chinese Hamster Ovary) identified a 152 amino acid soluble form with an N-terminal sequence beginning with the alanine residue at amino acid 134 (arrow in Figures 7A and 7B). Reconstruction of the mass to charge ratio defined a mass for Neutrokine-α of 17,038 Daltons, a value in compete agreement with that predicted for this 152 amino acid protein with a single disulfide bond (17037.5 Daltons).

The expression profiles of Neutrokine-α mRNA were assessed by Northern blot (Figure 7B) and flow cytometric analyses (Table III and Figures 8A and 8B). Neutrokine-α is encoded by a single 2.6kb mRNA found at high levels in peripheral blood leukocytes, spleen, lymph node and bone marrow. Lower expression levels were detectable in placenta, heart, lung, fetal liver, thymus and pancreas. Among a panel of cell lines, Neutrokine-α mRNA was detected in HL60 and K562, but not in Raji, HeLa, or MOLT-4 cells. These results were confirmed by flow cytometric analyses using the Neutrokine-α-specific mAb 12D6A. As shown in Table III, Neutrokine-α expression is not detected on T or B lineage cells but rather restricted to cells within the myeloid

lineage. Representative staining profiles of the tumor lines K562, HL-60, U937 and THP-1 cells with 12E6 are shown in Figure 8A. Further analyses of normal blood cell types demonstrated significant expression on resting monocytes that was upregulated approximately 4-fold following exposure of cells to IFNγ (100U/mL) for three days (Figure 8B). Neutrokine-a was not expressed on freshly isolated neutrophils, T cells, B cells, and NK.

To generate Neutrokine-a recombinant protein, Neutrokine-a encoding amino acids 112-285 were fused to a heterologous signal peptide and subcloned into a baculovirus expression vector. Recombinant Neutrokine-a was purified from 10 liters of recombinant baculovirus infected Sf9 cell supernatants at 92 h post-infection. The insect cells were grown in EXCEL401 medium (JRH Scientific) with 1% (v/v) fetal bovine serum. The harvested supernatant was clarified by centrifugation at 18,000 x g followed by 0.45 µm depth filtration.

The supernatant was loaded onto a set of porose HS-50/HQ-50 in tandem mode. The HS/HQ flow through material was adjusted to pH 7.5 with 1 M Tris-HCl pH 8, diluted with equal volume of 50 mM Tris-HCl pH 8, and loaded onto a poros PI-20 column. The PI column was washed first with 4 column volumes of 75 mM NaCl in 50 mM Tris-HCl at pH 7.5, then eluted using 3 to 5 column volumes of a stepwise gradient of 300 mM, 750 mM, 1500 mM sodium chloride in 50 mM Tris-HCl pH 7.5. Neutrokine-a protein appears as a 17 KD band on reduced SDS-PAGE and is present in the 0.3 M to 1.5M NaCl fractions.

The PI fraction was further purified through a Sephacryl S100 HR size exclusion column equilibrated with 0.15 M NaCl, 50 mM NaOAc at pH 6. The S200 fractions were mixed with NaCl to a final concentration of 3 M and loaded onto a Toyopearl Hexyl 650C column. The Hexyl column was eluted with a linear gradient from 3 M to 0.05 M NaCl in 50 mM NaOAc pH6 in 15 column volumes. Fractions containing purified Neutrokine-a as analyzed through SDS-PAGE were combined and dialyzed against a buffer containing 150 mM NaCl, 50 mM NaOAc.

The final purified Neutrokine-a protein has an N-terminus sequence of AVQGP (beginning with amino acid residue Ala-134 of SEQ ID NO:2). This corresponds identically to the sequence of soluble Neutrokine-a derived from CHO cells lines stably transfected with the full length Neutrokine-a gene. RP-HPLC analysis shows a single peak of greater than 95% purity. Endotoxin level was below the detection limit in LAL assay.

Purified rNeutrokine-a was assessed for its ability to induce activation, proliferation, differentiation or death in numerous cell based assays involving B

cells, T cells, monocytes, NK cells, hematopoietic progenitors, and a variety of cell types of endothelial and epithelial origin. Among these assays, Neutrokine-α was uniquely found to increase B cell proliferation in a standard co-stimulatory assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM as priming agents. As shown in Figure 9A, recombinant Neutrokine-α purified from baculovirus cultures induces a dose-dependent proliferation of tonsillar B cells. This response is qualitatively like that of rhuIL2 over the dose range from 0.1 to 10,000 ng/mL. Neutrokine-α also induces B cell proliferation when cultured with cells co-stimulated with immobilized anti-IgM (Figure 9B). A dose-dependent response is readily observed as the amount of crosslinking agent increases in the presence of a fixed concentration of either IL2 or rNeutrokine-α. As with SAC, the magnitude of the rNeutrokine-α response is approximately half that of IL2 at any given concentration.

To further define the structural and functional characteristics of Neutrokine-α, amino-truncated forms of the protein were generated. A total of 6 truncated proteins encoding various portions of the extracellular domain were transiently expressed in CHO cells with the resulting supernatants screened for biological activity in the standard B cell co-stimulatory SAC assay. As shown in Figure 10, the soluble forms of Neutrokine-α beginning at residue Ala-71, Ala-81, Leu-112, and Ala-134 (of SEQ ID NO:2) were equally active. In contrast, the activity associated with mutants beginning at Leu-147 and Gly-161 (of SEQ ID NO:2) were no different than that of supernatants obtained from cells transfected with the vector control (pC4). Taken together, it appears that the predicted beta-pleated sheet formed by amino acid residues 144-151 of SEQ ID NO:2 (see Figures 7A and 7B) is critical for effective B cell signaling.

The ability of Neutrokine-α to uniquely activate normal B cell populations predicts the existence of a specific cell surface receptor(s). In an attempt to assess Neutrokine-α receptor distribution and potentially define novel cellular targets, purified rNeutrokine-α was biotinylated using a N-hydroxysuccinimidobiotin reagent and associated protocols provided by the manufacturer (Pierce, Rockford, IL). The resultant biotin-Neutrokine-α protein retained function as it was equally effective at stimulating B cell proliferation when compared to unlabelled rNeutrokine-α. Direct binding of biotin-Neutrokine-α was assessed by Flow cytometric means using a strepavidin-phycoerythrin conjugate. Analyses indicate that the cellular receptor(s) for Neutrokine-α are expressed on normal and neoplastic cells of the B lineage

(Figure 11). Biotinylated Neutrokine-a bound freshly isolated tonsillar B cells in a dose dependent manner with saturating levels attained at approximately 10ng of labelled protein per 10^6 cells. Receptor expression was also detected on the myeloma cell line IM9 but the level of binding was significantly less at all concentrations tested raising the possibility of fewer receptors per cell, the presence of lower affinity receptor(s) or novel receptor(s). Lineage-specific analyses of whole human peripheral blood cells indicates that binding of biotinylated Neutrokine-a was undetectable on T cells, NK cells, monocytes and granulocytes as assessed by CD3, CD56, CD14, and CD66b respectively. In contrast, biotinylated Neutrokine-a bound B cells as defined by CD20 surface expression. Taken together, these assay technique suggest that Neutrokine-a dispays a clear B cell tropism in both its receptor distribution and biological activity. It remains possible however, that activation of these or other cell populations may induce expression of Neutrokine-a receptors that are not present on freshly isolated whole blood cells or established neoplastic cell lines.

In parallel experiments it was determined that rNeutrokine-a efficiently stimulated proliferation of mouse splenic B cells but not immature, Ig^- B cell precursors isolated from mouse bone marrow. These observations afforded the opportunity to test the *in vivo* activity of rNeutrokine-a in a responsive species. Accordingly, BALB/c mice (3/group) were injected (i.p.) twice per day with buffer only, or 2 mg/Kg of rNeutrokine-a. Mice received this treatment for 4 consecutive days at which time they were sacrificed and various tissues and serum collected for analyses. The effects of Neutrokine-a administration was evident histoligically in both H&E stained and CD45R(B220) stained sections (Figure 12A). Comparison of H&E sections from normal and Neutrokine-a-treated spleens identified diffuse peri-arterial lymphatic sheaths and a significant increase in the nucleated cellularity of the red pulp regions (Figure 12A). Immunohistochemical studies using a B cell marker, anti-CD45R(B220) suggest that the splenic disorganization observed in Neutrokine-a treated mice was due to increased B cell representation within loosely defined B cell zones that infiltrated established T cell regions. Further experiments will be required to define the mechanism by which Neutrokine-a alter spenic architecture.

Flow cytometric analyses of the spleens from Neutrokine-a treated mice indicate that Neutrokine-a specifically increased the proportion of ThB+, CD45R(B220)dull B cells over that observed in control mice (Figure 11). The increase was greater than 10-fold in mice.

A predicted consequence of increased mature B cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA

levels were compared between buffer and Neutrokine-a-treated mice. Neutrokine-a administration resulted in a 2 to 5-fold increase in both IgM and IgA serum levels.

Discussion

The data presented herein describes Neutrokine-a, a novel member of the TNF-ligand superfamily that specifically induces both *in vivo* and *in vitro* B cell proliferation and differentiation. The biological profile of Neutrokine-a is unique based on its restricted gene/protein expression and its apparent B cell tropism. The potential uses of such a factor, its receptor(s) or functionally related agonists and antagonists of either are diverse. These agents may find application as diagnostic and/or therapeutic agent in virtually any aspect of the normal and diseased immune system.

Finally, the chromosomal location of Neutrokine-a was determined using a combination of somatic cell hybrids and radiation hybrids to chromosome position 13q34. This is the first member of the TNF cytokine superfamily to map to this region. Knowing the genomic location of Neutrokine-a and its biological profile allows one to correlate specific inherited disorders with potential alterations in Neutrokine-a, its associated receptor(s) and/or related signalling pathways.

The assays and experiments described above clearly provide the scientific rational for the use of Neutrokine-a as a regulator of B cell proliferation and differentiation. The possible uses of the either soluble of membrane bound Neutrokine-a, its native receptor and various receptor antagonists are diverse and include treatement of autoimmune disorders and immunodeficiencies resulting from infection, anti-neoplastic therapy and/or inherited disorders. Moreover, many of the pre-neoplastic monoclonal gammopathies and neoplastic B cell diseases such as multiple myeloma may utilize Neutrokine-a or its receptor as either inducing or progressing factors.

Accordingly, Neutrokine-a or derived, functional agonists may find application as the following:

A vaccine adjuvant that enhances immune responsiveness to specific antigen.

An adjuvant to enhance tumor-specific immune responses.

An adjuvant to enhance anti-viral immune responses.

As a stimulator of B cell responsiveness to pathogens.

As an agent that elevates the immune status of a individual prior to their receipt of immunosuppressive therapies.

As an agent to accelerate recovery of immunocompromised individuals;

As an agent to boost immunoresponsiveness among aged populations;

As an immune system enhancer following bone marrow transplant.

As a mediator of mucosal immune responses. The expression of Neutrokine-a by monocytes and the responsiveness of B cell to this factor suggests that it may be involved in exchange of signals between B cells and monocytes or their differentiated progeny. This activity is in many ways analogous to the CD40-CD154 signalling between B cells and T cells. Neutrokine-a may therefore be an important regulator of T cell independent immune responses to environmental pathogens. In particular, the unconventional B cell populations (CD5+) that are associated with mucosal sites and responsible for much of the innate immunity in humans may respond to Neutrokine-a thereby enhancing an individual's protective immune status.

As an agent to direct an individuals immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

As B cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic B cell populations.

As a means of detecting B-lineage cells by virtue of its specificity. This application may require labeling the protein with biotin or other agents to afford a means of detection.

As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodificiency;

As part of a B cell selection device the function of which is to isolate B cells from a heterogenous mixture of cell types. Neutrokine-a could be coupled to a solid support to which B cells would then specifically bind. Unbound cells would be washed out and the bound cells subsequently eluted. This technique would allow purging of tumor cells from, for example, bone marrow or peripheral blood prior to transplant.

As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

As an antigen for the generation of antibodies to inhibit or enhance Neutrokine-a mediated responses.

As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.

As a means of regulating secreted cytokines that are elicited by Neutrokine-a.

All of the above described applications as they may apply to veterinary medicine.

Antagonists of Neutrokine-a include binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the Neutrokine-a receptor(s). These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens. Although our current data speaks directly to the potential role of Neutrokine-a in B cell and monocyte related pathologies, it remains possible that other cell types may gain expression or responsiveness to Neutrokine-a. Thus, Neutrokine-a may, like CD40 and its ligand, be regulated by the status of the immune system and the microenvironment in which the cell is located.

A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythramatosus and MS.

An inhibitor of graft versus host disease or transplant rejection.

A therapy for B cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.

A therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, and related idiopathic monoclonalgammopathies.

A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

An immunosuppressive agent(s).

An inhibitor of signalling pathways involving ERK1, COX2 and Cyclin D2 which have been associated with Neutrokine-a induced B cell activation.

Isolation of a cDNA showing homology to the TNF ligand superfmaily

An expressed sequence tag (EST) database of human cDNAs was screened for homologs of TNF alpha utilizing the Neutrokine-a algorithiom. Several overlapping ESTs showing homology to TNF and other family members were identified, and the longest clone (isolated from a neutrophil library) was picked and the full length sequence determined. The designated methionine is likely to be the start codon as there is an upstream in frame stop codon and no upstream in frame methionines.

Mammalian Cell transfections

Cell culture reagents obtained from Life Technologies. Human embryonic kidney cells 293 were maintained in DMEM containing 10% serum

Purification of recombinant human Neutrokine-a (polypeptide fragment from Ala-134 to Leu-285 of SEQ ID NO:2).

To generate Neutrokine-a recombinant protein, Neutrokine-a encoding amino acid residues 112 through 285 of SEQ ID NO:2 was fused to a heterologous signal peptide and subcloned into a baculovirus expression vector. Recombinant Neutrokine-a was purified from 10 liters of recombinant baculovirus infected Sf9 cell supernatants at 92 h post-infection. The insect cells were grown in EXCEL401 medium (JRH Scientific) with 1% (v/v) fetal bovine serum. The harvested supernatant was clarified by centrifugation at 18,000 x *g* followed by 0.45 µm depth filtration.

The supernatant was loaded onto a set of porose HS-50/HQ-50 in tandem mode. The HS/HQ flow through material was adjusted to pH 7.5 with 1 M Tris-HCl pH 8, diluted with equal volume of 50 mM Tris-HCl pH 8, and loaded onto a poros PI-20 column. The PI column was washed first with 4 column volumes of 75 mM NaCl in 50 mM Tris-HCl at pH 7.5, then eluted using 3 to 5 column volumes of a stepwise gradient of 300 mM, 750 mM, 1500 mM sodium chloride in 50 mM Tris-HCl pH 7.5. Neutrokine-a protein appears as a 17 KD band on reduced SDS-PAGE and is present in the 0.3 M to 1.5M NaCl fractions.

The PI fraction was further purified through a Sephacryl S100 HR size exclusion column equilibrated with 0.15 M NaCl, 50 mM NaOAc at pH 6. The

S200 fractions were mixed with NaCl to a final concentration of 3 M and loaded onto a Toyopearl Hexyl 650C column. The Hexyl column was eluted with a linear gradient from 3 M to 0.05 M NaCl in 50 mM NaOAc pH6 in 15 column volumes. Fractions containing purified TL7 as analyzed through SDS-PAGE were combined and dialyzed against a buffer containing 150 mM NaCl, 50 mM NaOAc.

The final purified Neutrokine-a protein has an N-terminus sequence beginning with Ala-134 of SEQ ID NO:2 (AVQGP). This corresponds identically to the sequence of soluble Neutrokine-a derived from CHO cells lines stably transfected with the full length Neutrokine-a gene. RP-HPLC analysis shows a single peak of greater than 95% purity. Endotoxin level was below the detection limit in LAL assay.

Northern Blot Analysis

Northern blot analysis was performed utilizing the following membranes: human multiple tissue Northern blots I and II, a human cancer cell line blot, and an Immune blot of poly(A) RNA (2 ug, Clonetech). Blots were hybridized with random-primed ^{32}P-labeled probes according to manafacturer's recommendations. As a probe the complete Neutrokine-a (SEQ ID NO:1) cDNA was used.

Chromosomal Mapping

To determine the chromosomal location of the Neutrokine-a gene, a panel of monochromosomal somatic cell hybrids (obtained from Quantum Biotechnology) retaining individual chromosomes was screened by PCR using Neutrokine-a specific primers. The following oligonucleotides which span a 233 base pair region of the Neutrokine-a coding region were used for PCR analysis on 100 ng of template DNA: TGGTGTCTTTCTACCAGGTGG (5' primer); TTTCTTCTGGACCCTGAACGG (3' primer). 35 cycles of PCR amplification (94°C - 30 secs; 58°C - 45 secs; 72°C - 1 min) were performed on 100 ng of each hybrid in a 50 ul reaction. The anticipated 233 bp PCR product was detected in human chromosome 13, while no amplification was observed in any other sample. To sublocalize Neutrokine-a on chromosome 13, a panel of 83 radiation hybrids (obtained from Research Genetics) was used. In addition to the human genomic DNA, amplicons were observed in hybrids 4, 8, 21, 36, 51, 58, 64, 66 and 75. Analysis of this data using the Stanford Human Genome Center RHserver revealed linkage to the SHGC-36171 marker on chromosome 13. Superposition of this map with the cytogenetic map of human

chromosome 13 allowed the assignment of Neutrokine-a to chromosomal band 13q34. Analysis of the radiation hybrid data was performed using the server at the Stanford Human Genome Center

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

Further, the Sequence Listing submitted herewith, and the Sequence Listings submitted in copending application Serial Nos. 09/005,874, filed January 12, 1998, US60/036,100, filed January 14, 1997, and PCT/US96/17957, filed October 25, 1996, in both computer and paper forms in each case, are hereby incorporated by reference in their entireties.

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the Neutrokine-a polypeptide having the complete amino acid sequence in Figures 1A and 1B (SEQ ID NO:2);

(b) a nucleotide sequence encoding the Neutrokine-a polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 97768;

(c) a nucleotide sequence encoding the Neutrokine-a polypeptide extracellular domain;

(d) a nucleotide sequence encoding the Neutrokine-a polypeptide transmembrane domain;

(e) a nucleotide sequence encoding the Neutrokine-a polypeptide intracellular domain;

(f) a nucleotide sequence encoding a soluble Neutrokine-a polypeptide comprising the extracellular and intracellular domains but lacking the transmembrane domain; and

(g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above.

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figures 1A and 1B (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figures 1A and 1B (SEQ ID NO:1) encoding the Neutrokine-a polypeptide having the complete amino acid sequence in Figures 1A and 1B (SEQ ID NO:2).

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding a soluble Neutrokine-a polypeptide comprising the extracellular domain shown in Figures 1A and 1B (SEQ ID NO:2).

5. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues n-285 of SEQ ID NO:2, where n is an integer in the range of 2-190

(b) a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues 1-m of SEQ ID NO:2, where m is an integer in the range of 274-284;

(c) a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues n-m of SEQ ID NO:2, where n and m are integers as defined respectively in (a) and (b) above; and

(d) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete Neutrokine-a amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 97768, wherein said portion excludes from 1 to 190 amino acids from the amino terminus and from 1 to 11 amino acids from the C-terminus of said complete amino acid sequence.

6. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in the deposit having ATCC accession number 97768.

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the Neutrokine-a polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 97768.

8. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding a soluble Neutrokine-a polypeptide comprising the extracellular domain encoded by the cDNA clone contained in the deposit having ATCC accession number 97768.

9. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e) or (f) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

10. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a Neutrokine-a polypeptide having an amino acid sequence in (a), (b), (c), (d), (e) or (f) of claim 1.

11. The isolated nucleic acid molecule of claim 10, which encodes an epitope-bearing portion of a Neutrokine-a polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about Phe-115 to about Leu-147 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ile-150 to about Tyr-163 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ser-171 to about Phe-194 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Glu-223 to about Tyr-247 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about Ser-271 to about Phe-278 (SEQ ID NO:2).

12. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

13. A recombinant vector produced by the method of claim 12.

14. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 13 into a host cell.

15. A recombinant host cell produced by the method of claim 14.

16. A recombinant method for producing a Neutrokine-a polypeptide, comprising culturing the recombinant host cell of claim 15 under conditions such that said polypeptide is expressed and recovering said polypeptide.

17. An isolated Neutrokine-a polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the Neutrokine-a polypeptide having the complete amino acid sequence in Figures 1A and 1B (SEQ ID NO:2);

(b) the amino acid sequence of the Neutrokine-a polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 97768;

(c) the amino acid sequence of the Neutrokine-a polypeptide extracellular domain;

(d) the amino acid sequence of the Neutrokine-a polypeptide transmembrane domain;

(e) the amino acid sequence of the Neutrokine-a polypeptide intracellular domain;

(f) the amino acid sequence of a soluble Neutrokine-a polypeptide comprising the domain; and

(g) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e) or (f).

18. An isolated polypeptide of claim 17 comprising an epitope-bearing portion of the Neutrokine-a protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about Phe-115 to about Leu-147 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ile-150 to about Tyr-163 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ser-171 to about Phe-194 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Glu-223 to about Tyr-247 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ser-271 to about Phe-278 (SEQ ID NO:2).

19. An isolated antibody that binds specifically to a Neutrokine-a polypeptide of claim 17.

20. A pharmaceutical composition comprising a polypeptide of claim 17 and a pharmaceutically acceptable carrier.

21. An isolated polynucleotide encoding a modified Neutrokine-a protein, wherein, except for at least one conservative amino acid substitution, said modified peptide has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids 1 to 285 of SEQ ID NO:2;
(b) amino acids 2 to 285 of SEQ ID NO:2;
(c) amino acids 1 to 46 of SEQ ID NO:2;
(c) amino acids 47 to 72 of SEQ ID NO:2; and
(c) amino acids 73 to 286 of SEQ ID NO:2.

22. A modified Neutrokine-a polypeptide molecule, wherein, except for at least one conservative amino acid substitution, said modified peptide has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids 1 to 285 of SEQ ID NO:2;
(b) amino acids 2 to 285 of SEQ ID NO:2;
(c) amino acids 1 to 46 of SEQ ID NO:2;
(c) amino acids 47 to 72 of SEQ ID NO:2; and
(c) amino acids 73 to 286 of SEQ ID NO:2.

23. An isolated nucleic acid molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:7;
(b) the nucleotide sequence of SEQ ID NO:8;
(c) the nucleotide sequence of SEQ ID NO:9;
(d) the nucleotide sequence of a portion of the sequence shown in Figures 1A and 1B (SEQ ID NO:1) wherein said portion comprises at least 30 contiguous nucleotides from nucleotide 1 to nucleotide 2442, excluding the sequence from nucleotide 1387 to 1421, the sequence from nucleotide 9 to 382, the sequence from nucleotide 1674 to 1996, the sequence from nucleotide 1401 to 1784, the sequence from nucleotide 900 to 1237, and any fragments located within these sequences; and
(e) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c) or (d) above.

24. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the Neutrokine-aSV polypeptide having the complete amino acid sequence in Figures 5A and 5B (SEQ ID NO:19);

(b) a nucleotide sequence encoding the Neutrokine-aSV polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 203518;

(c) a nucleotide sequence encoding the Neutrokine-aSV polypeptide extracellular domain;

(d) a nucleotide sequence encoding the Neutrokine-aSV polypeptide transmembrane domain;

(e) a nucleotide sequence encoding the Neutrokine-aSV polypeptide intracellular domain;

(f) a nucleotide sequence encoding a soluble Neutrokine-aSV polypeptide comprising the extracellular and intracellular domains but lacking the transmembrane domain; and

(g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above.

Abstract

The present invention relates to a novel Neutrokine-a, and a splice variant thereof designated Neutrokine-aSV, polynucleotides and polypeptides which are members of the TNF family. In particular, isolated nucleic acid molecules are provided encoding the human Neutrokine-a and/or Neutrokine-aSV polypeptides, including soluble forms of the extracellular domain. Neutrokine-a and/or Neutrokine-aSV polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of Neutrokine-a and/or Neutrokine-aSV activity. Also provided are diagnostic methods for detecting immune system-related disorders and therapeutic methods for treating immune system-related disorders.

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I declare that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Neutrokine-α and Neutrokine-α Splice Variant

the specification of which is attached hereto, or was filed on _____ as Application Serial No. _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed Yes

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

60/036,100	January 14, 1997
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(b) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

09/005,874	January 12, 199	Pending
(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)
PCT/US96/17957	October 25, 1996	Pending
(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Robert H. Benson (Reg. No. 30,446), A. Anders Brookes (Reg. No. 36,373), James H. Davis (Reg. No. 40,582) and Kenley K. Hoover (Reg. No. 40,302) of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20878.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Table III: *Cell surface expression of Neutrokine-alpha as detected by mAb 12E6*

Cell line	Cellular Morphology	Neutrokine-alpha cell surface expression
Monocytic lineage		
U937	Lymphoma, histiocytic/macrophage	+
HL-60	Leukemia, acute promyelocytic	+
K562	Leukemia, chronic myelogenous	+
THP-1	Leukemia, acute monocytic	+
T-lineage		
Jurkat	Leukemia, T lymphocytic	-
MOLT-4	Leukemia, T lymphoblastic	-
B-lineage		
Daudi	Burkitt's, lymphoblastic	-
Namalwa	Burkitt's, lymphocyte	-
Raji	Burkitt's, lymphocyte	-
Reh	Leukemia, lymphocytic	-
ARH-77	Leukemia, plasma cell	-
IM-9	Myeloma	-
RPMI 8226	Myeloma	-

Figure 1A
Neutrokine-α

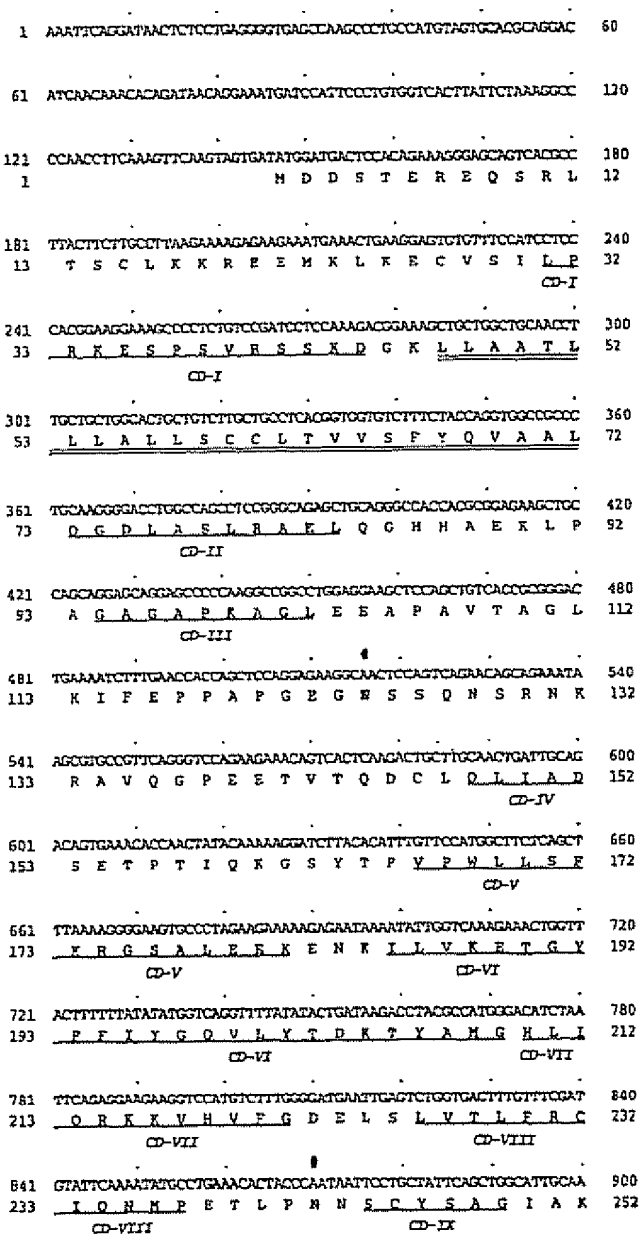


Figure 1B
Neutrokine-α

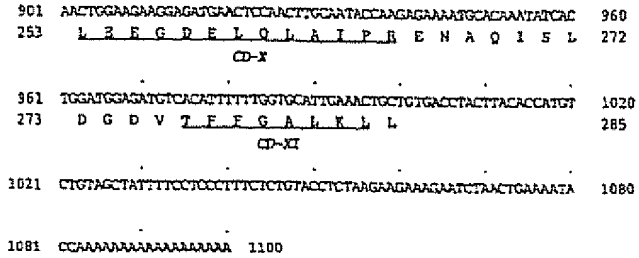


FIGURE 2A

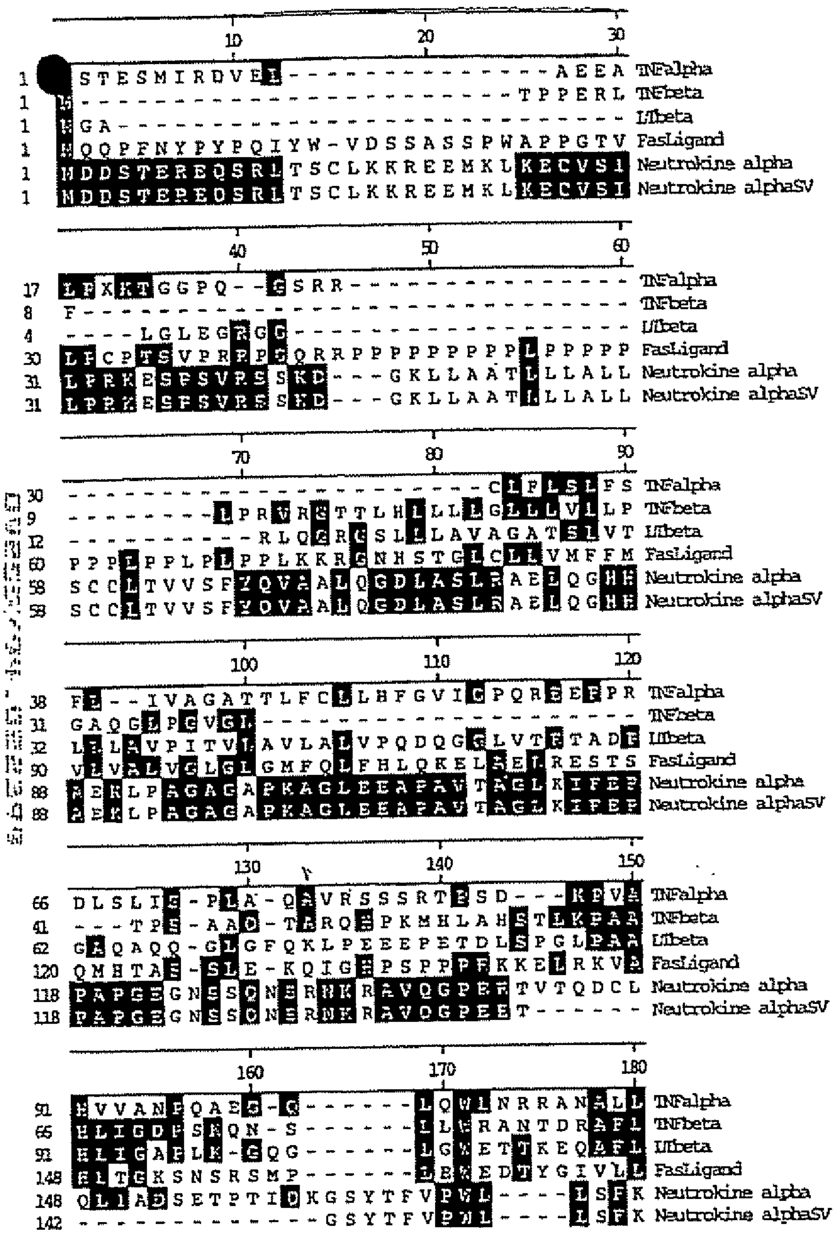
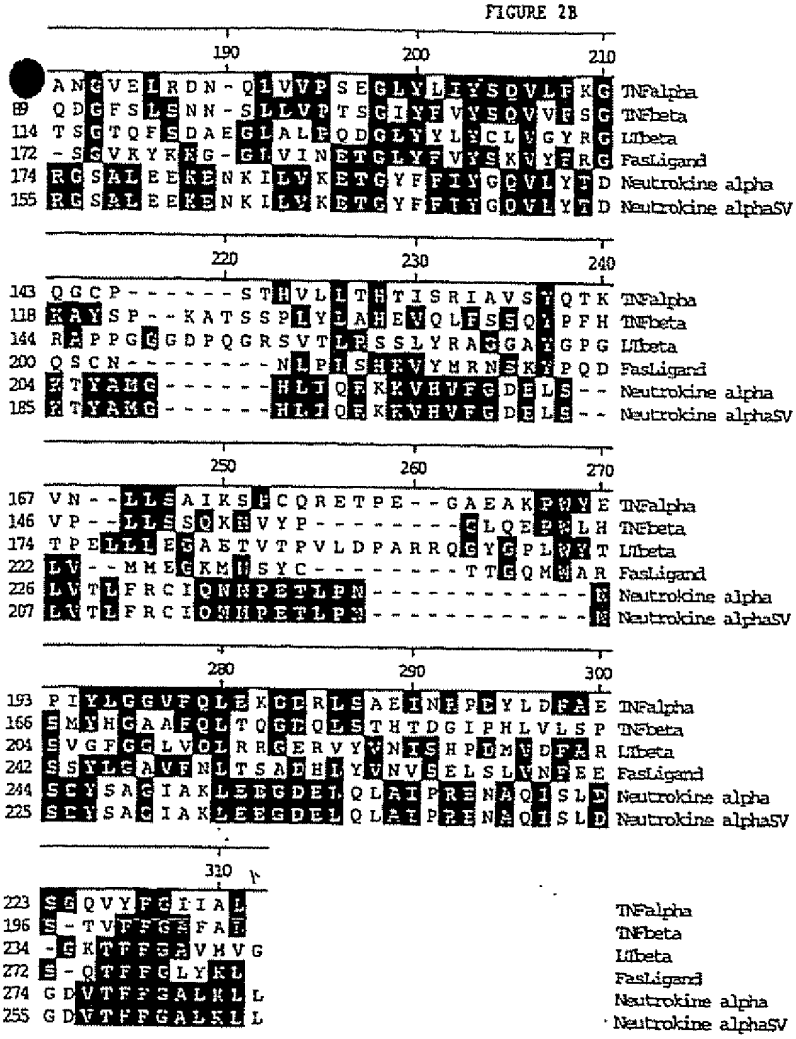

TNFalpha
TNFbeta
LTbeta
FasLigand
Neutrokine alpha
Neutrokine alphaSV

FIGURE 2B



Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Figure 3
Neutrokine-α

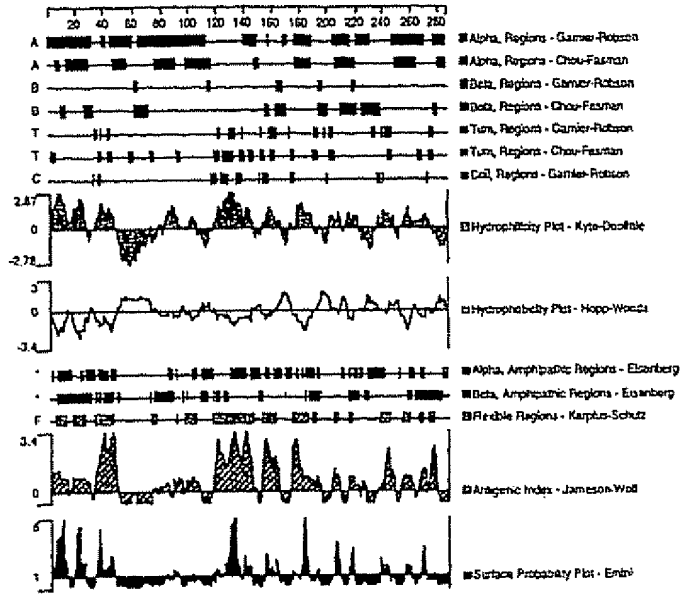


FIGURE 4 A

```
          1                                                        50
HSOAD55R  .........A GGNTAACTCT CCTGAGGGGT GAGCCAAGCC CTGCCATGTA
HNEDU15X  ...AAATTCA GGATAACTCT CCTGAGGGGT GAGCCAAGCC CTGCCATGTA
HSLAH84R  .AATTCGGCA NAGNAAACTG GTTACTTTTT TATATATGGT CAGGTTTTAT
HLTBM08R  AATTCGGCAC GAGCAAGGCC GGCCTGGAGG AAGCTCCAGC TGTCACCGCG

          51                                                      100
HSOAD55R  GTGCACGCAG GACATCANCA A..ACACANN NNNCAGGAAA TAATCCATTC
HNEDU15X  GTGCACGCAG GACATCAACA A..ACACAGA TAACAGGAAA TGATCCATTC
HSLAH84R  ATACTGATAA GACCTACGCC ATGGGACATC TAGTTCAGAG GAAGAAGGTC
HLTBM08R  GGACTGAAAA TCTTTGAACC ACCAGCTCCA GGAGAAGGCA ACTCCAGTCA

          101                                                     150
HSOAD55R  CCTGTGGTCA CTTATTCTAA AGGCCCCAAC CTTCAAAGTT CAAGTAGTGA
HNEDU15X  CCTGTGGTCA CTTATTCTAA AGGCCCCAAC CTTCAAAGTT CAAGTAGTGA
HSLAH84R  CATGTCTTTG GGGATGAATT GAGTCTGGTG ACTTTGTTTC GATGTATTCA
HLTBM08R  GAACAGCAGA AATAAGCGTG CCGTTCAGGG TCCAGAAGAA ACAGTCACTC

          151                                                     200
HSOAD55R  TATGGATGAC TCCACAGAAA GGGAGCAGTC ACGCCTTACT TCTTGCCTTA
HNEDU15X  TATGGATGAC TCCACAGAAA GGGAGCAGTC ACGCCTTACT TCTTGCCTTA
HSLAH84R  AAATATGCCT GAAACACTAC CCAATAATTC CTGCTATTCA GCTGGCATTG
HLTBM08R  AAGACTGCTT GCAACTGNTT GCAGACAGTG AAACACCAAC TATACAAAAA

          201                                                     250
HSOAD55R  AGAAAAGAGA AGAAATGAAA CTGNAAGGAG TGTGTTTCCA TCCTCCCACG
HNEDU15X  AGAAAAGAGA AGAAATGAAA CT.GAAGGAG TGTGTTTCCA TCCTCCCACG
HSLAH84R  CAAAACTGGN AGGAAGGA.. ...GATGAAC TCCAACTTGC AATACCAGGG
HLTBM08R  GGCTCCCTTC TGNTGCCACA TTTGGGCCAA GGAATGGAGA GATTTCTTCG

          251                                                     300
HSOAD55R  GAAGGAAAGC CCCTCTNTCC GATCCTCCAA AGACGGAAAG CTGCTGGCTG
HNEDU15X  GAAGGAAAGC CCCTCTGTCC GATCCTCCAA AGACGGAAAG CTGCTGGCTG
HSLAH84R  GAAAATGCAC AATTATCACT GGGATGGAGA TGTTCACATT TTTTGGGTGC
HLTBM08R  TCTGGAAACA TTTTGCCAAA CTCTTCAGAT ACTCTTTNCT CTCTGGGAAT

          301                                                     350
HSOAD55R  CAACCTTGNT GNTGGCATTG TGTTCTTGCT GNCTCAAGGT GGTGTTNTT.
HNEDU15X  CAACCTTGCT GCTGGCACTG CTGTCTTGCT GCCTCACGGT GGTGTCTTTC
HSLAH84R  CATTGAAACT GCTGTGACCT NCTTACANCA NGTGCTGTTN GCTATTTTNC
HLTBM08R  CAAAGGAAAA TCTCTACTTA GATTNACACA TTTGTTCCCA TGGGTNTCTT

          351                                                     400
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TACCAGGTGG CCGCCCTGCA AGGGGACCTG GCCAGCCTCC GGGCAGAGCT
HSLAH84R  CTNCCTNTTC TNTGGTAACC TCTTAGGAAG GAAGGATTCT TAACTGGGAA
HLTBM08R  AAGTTTTAAA AGGGGAGTGC CCTTAGGAGG AAAAGGGGAT AAATATTGGC
```

FIGURE 4B

```
          401                                                  450
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  GCAGGGCCAC CACGCGGAGA AGCTGCCAGC AGGAGCAGGA GCCCCCAAGG
HSLAH84R  ATAACCCAAA AAAANNTTAA ANGGGTANGN GNNANANGNG GGGNNGTTNN
HLTBM08R  CAAGGNACTG GTTANTTTNT AAATATGGTC AGGTTTNTAT ANCTGGTAGG

          451                                                  500
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CCGGCCTGGA GGAAGCTCCA GCTGTCACCG CGGGACTGAA AATCTTTGAA
HSLAH84R  CNNGNNGNNT TTTNGGNNTA TNTTNTNNTN GGGNNNNGTA AAAATGGGGC
HLTBM08R  CCTCGCCATG GGCATTNATT CANGGNGAGG NCNNTCTTTT GGGNTGA...

          501                                                  550
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CCACCAGCTC CAGGAGAAGG CAACTCCAGT CAGAACAGCA GAAATAAGCG
HSLAH84R  CNANGGGGGN TTTTT..... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          551                                                  600
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TGCCGTTCAG GGTCCAGAAG AAACAGTCAC TCAAGACTGC TTGCAACTGA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          601                                                  650
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TTGCAGACAG TGAAACACCA ACTATACAAA AAGGATCTTA CACATTTGTT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          651                                                  700
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CCATGGCTTC TCAGCTTTAA AAGGGGAAGT GCCCTAGAAG AAAAAGAGAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          701                                                  750
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TAAAATATTG GTCAAAGAAA CTGGTTACTT TTTTATATAT GGTCAGGTTT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          751                                                  800
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TATATACTGA TAAGACCTAC GCCATGGGAC ATCTAATTCA GAGGAAGAAG
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........
```

FIGURE 4C

```
          801                                                       850
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  GTCCATGTCT TTGGGGATGA ATTGAGTCTG GTGACTTTGT TTCGATGTAT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          851                                                       900
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TCAAAATATG CCTGAAACAC TACCCAATAA TTCCTGCTAT TCAGCTGGCA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          901                                                       950
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  TTGCAAAACT GGAAGAAGGA GATGAACTCC AACTTGCAAT ACCAAGAGAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

          951                                                      1000
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  AATGCACAAA TATCACTGGA TGGAGATGTC ACATTTTTTG GTGCATTGAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

         1001                                                      1050
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  ACTGCTGTGA CCTACTTACA CCATGTCTGT AGCTATTTTC CTCCCTTTCT
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

         1051                                                      1100
HSOAD55R  .......... .......... .......... .......... ..........
HNEDU15X  CTGTACCTCT AAGAAGAAAG AATCTAACTG AAAATACCAA AAAAAAAAAA
HSLAH84R  .......... .......... .......... .......... ..........
HLTBM08R  .......... .......... .......... .......... ..........

         1101
HSOAD55R  ......
HNEDU15X  AAAAAA
HSLAH84R  ......
HLTBM08R  ......
```

Figure 5A
Neutrokine-αSV

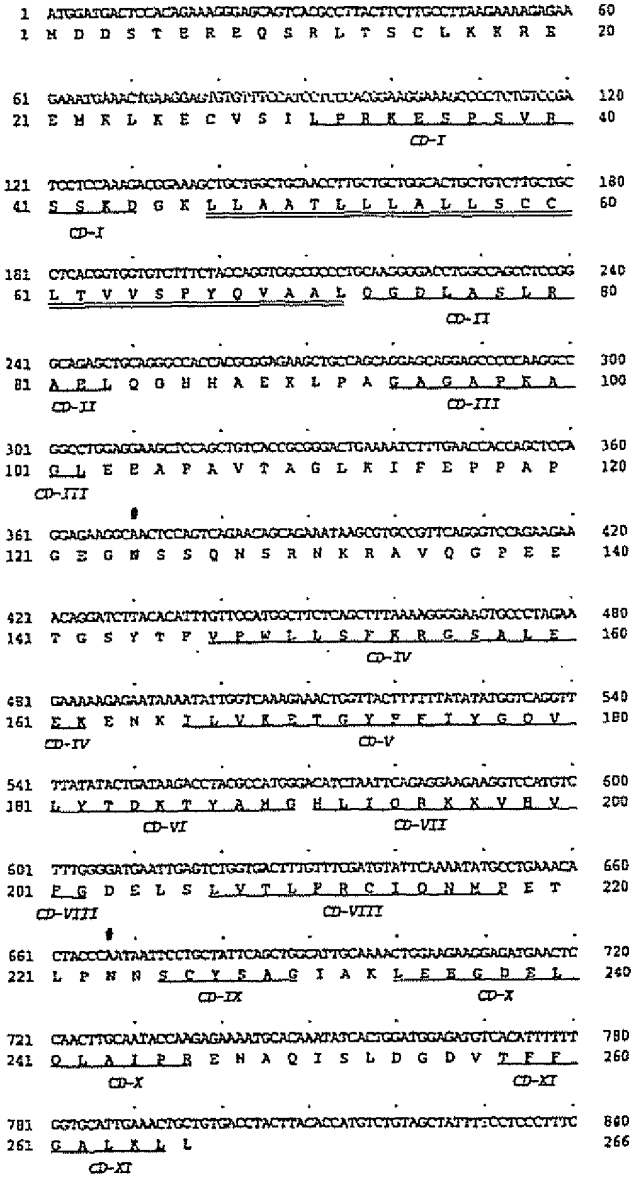


Figure 5B
Neutrokine-αSV

841 TCTGTACCTCTAAGAAGAAAGAATCTAACTGAAAATACCAAAAAAAAAAAAAAAAAAAAA 900

901 AAA 903

Figure 6
Neutrokine-αSV

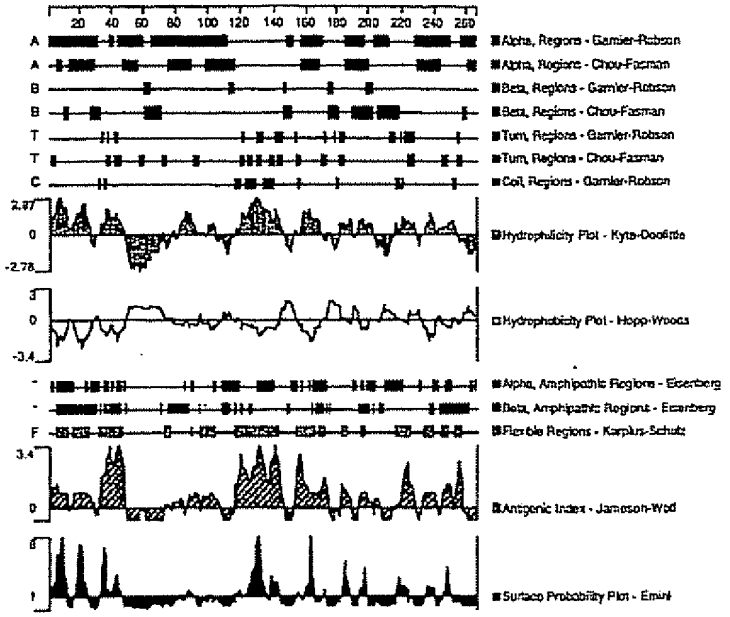


Figure 7A

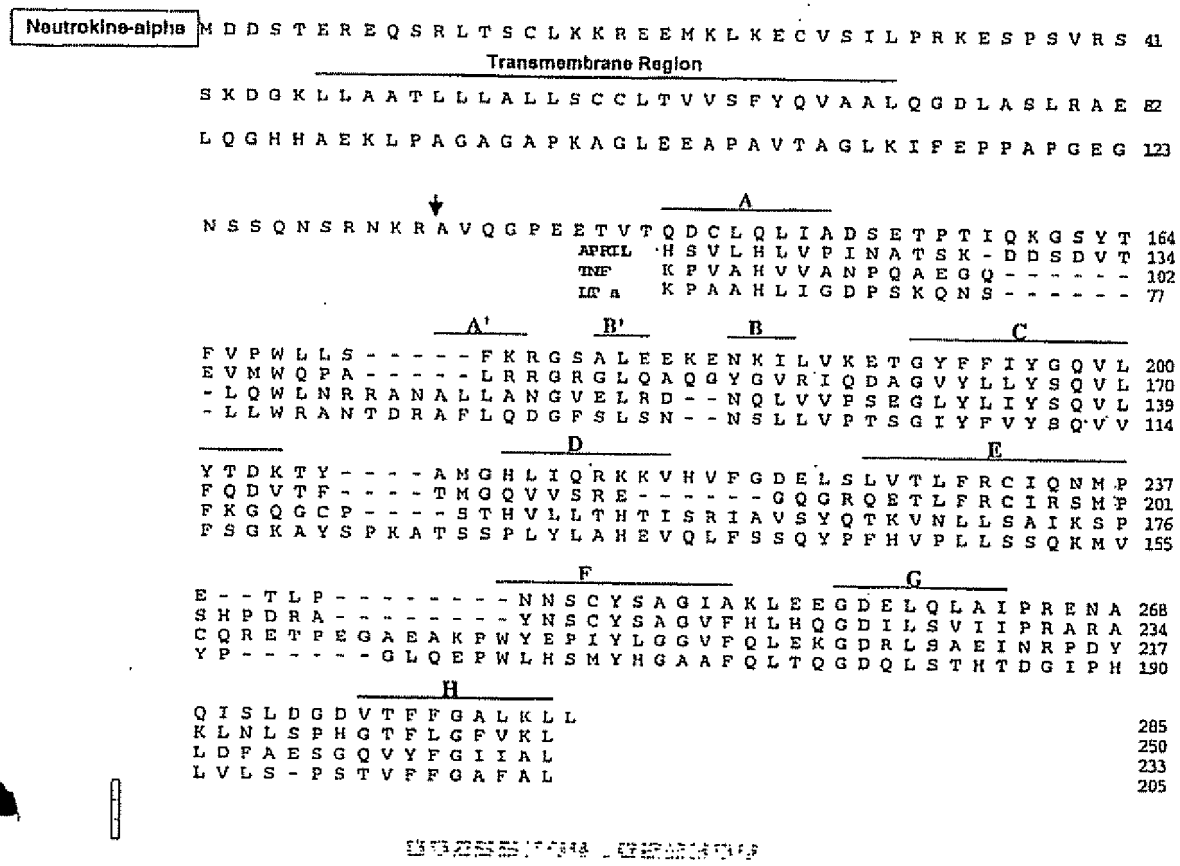

Neutrokine-alpha
Transmembrane Region
APRIL
TNF
LT a
A
A'
B'
B
C
D
E
F
G
H

Figure 7B

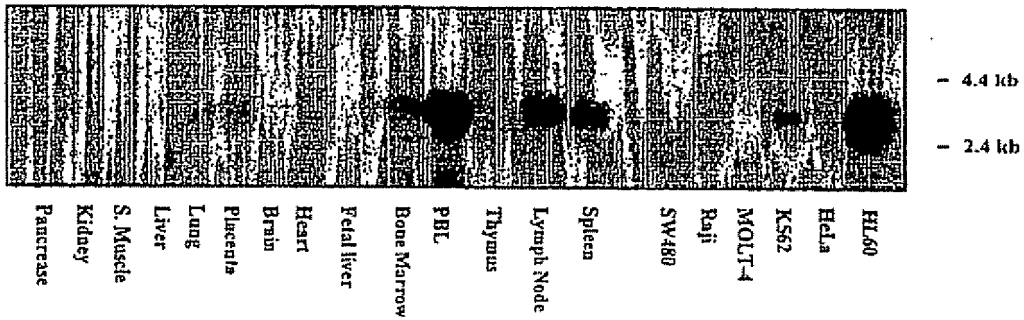
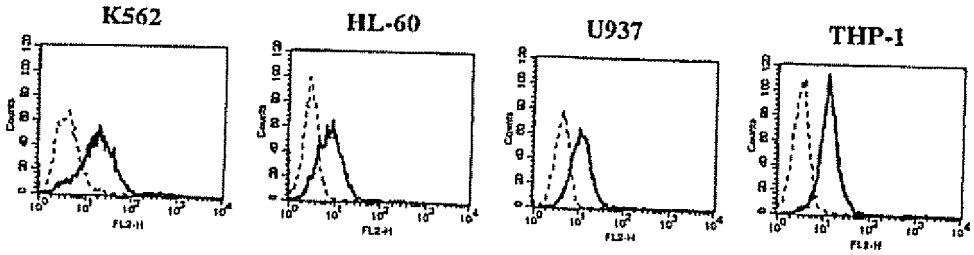

4.4 kb
2.4 kb
HL60
HeLa
K562
MOLT-4
Raji
SW480
Spleen
Lymph Node
Thymus
PBL
Bone Marrow
Fetal liver
Heart
Brain
Placenta
Lung
Liver
S. Muscle
Kidney
Pancrease

Figure 8.

A.


K562
HL-60
U937
THP-1
Counts
FL2-H

B.

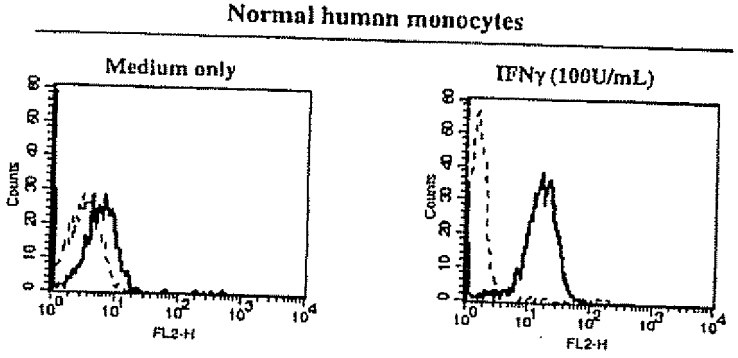
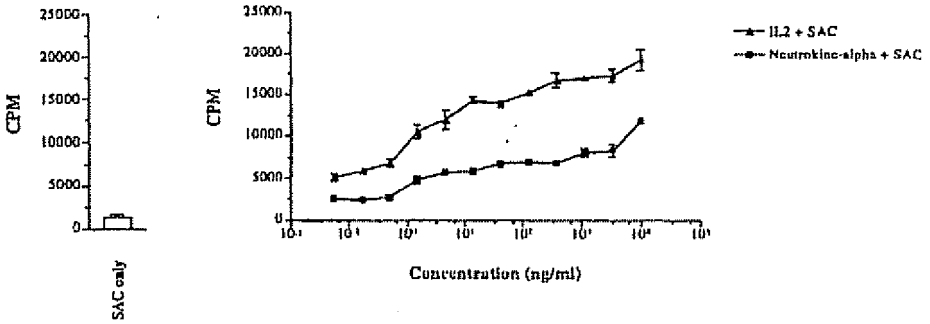

Normal human monocytes
Medium only
IFNγ (100U/mL)
Counts
FL2-H

Figure 9

A.


CPM
25000
20000
15000
10000
5000
0
SAC only
CPM
25000
20000
15000
10000
5000
0
Concentration (ng/ml)
IL2 + SAC
Neutrokine-alpha + SAC

B.

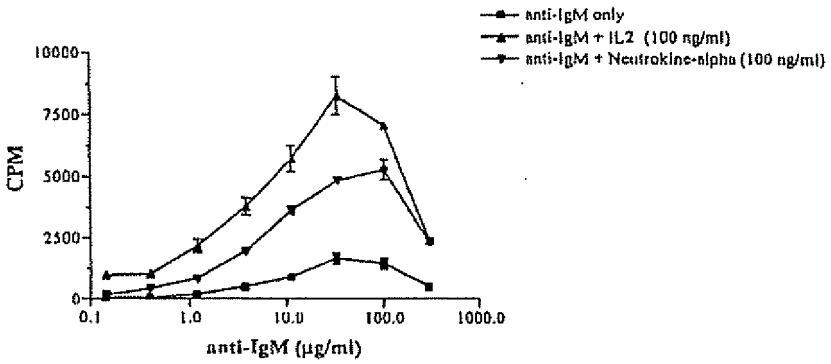

anti-IgM only
anti-IgM + IL2 (100 ng/ml)
anti-IgM + Neutrokine-alpha (100 ng/ml)
CPM
10000
7500
5000
2500
0
0.1
1.0
10.0
100.0
1000.0
anti-IgM (µg/ml)

Figure 10

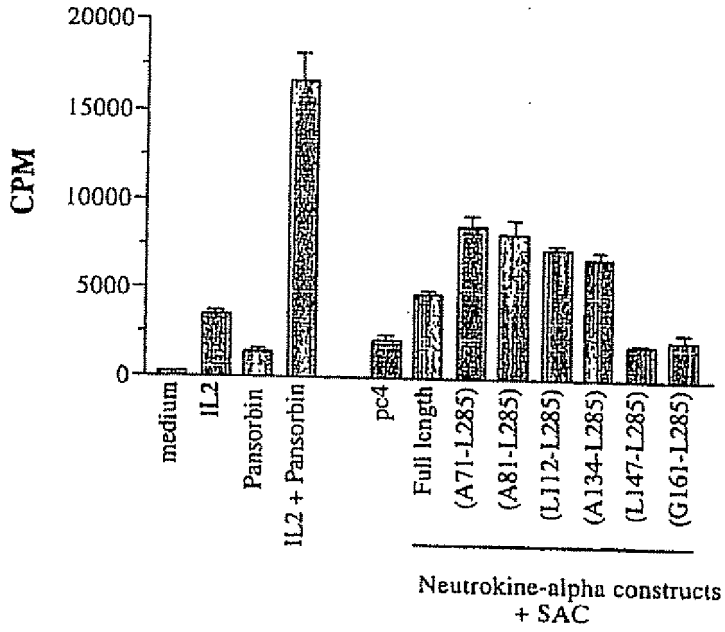
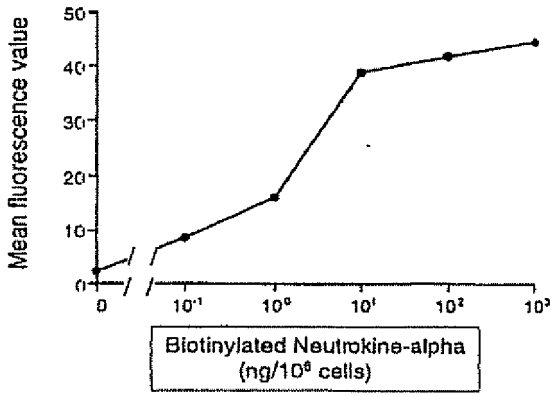

20000
15000
10000
5000
0
CPM
medium
IL2
Pansorbin
IL2 + Pansorbin
pc4
Full length
(A71-L285)
(A81-L285)
(L112-L285)
(A134-L285)
(L147-L285)
(G161-L285)
Neutrokine-alpha constructs
+ SAC

Figure 11

Tonsillar B cells



IM9 tumor cells

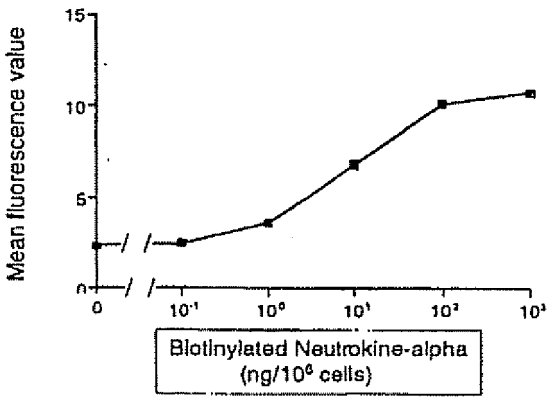


Figure 12A

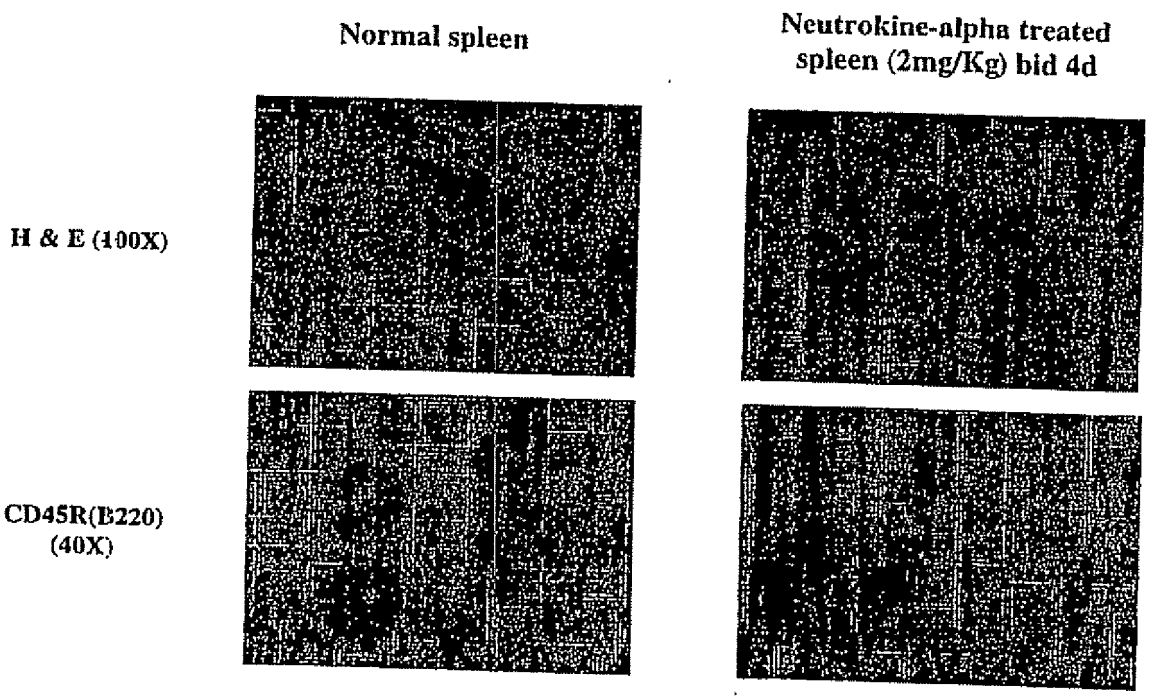
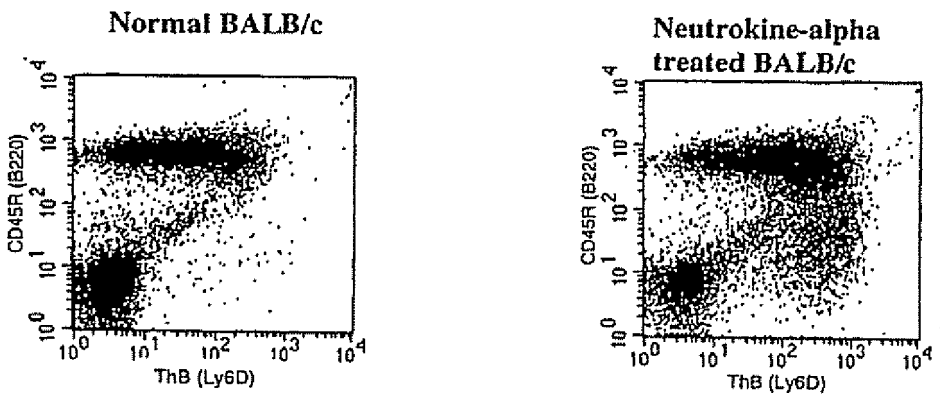

Normal spleen
Neutrokine-alpha treated spleen (2mg/Kg) bid 4d
H & E (100X)
CD45R(B220) (40X)

Figure 12B


Normal BALB/c
Neutrokine-alpha treated BALB/c
CD45R (B220)
ThB (Ly6D)
10^0
10^1
10^2
10^3
10^4